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THE ANALYSIS OF TRACE ORGANICS IN FRESH WATER BY GAS CHROMATOGRAPHY - MASS SPECTROSCOPY

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by

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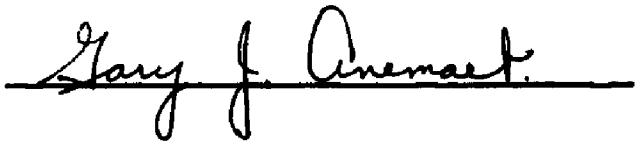

This thesis is dedicated to the author's wife,

DONNA

Her constant encouragement and understanding has made it possible to complete this work.

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ABSTRACT

A method for the qualitative analysis of organic substances in fresh water is presented. Four liters of water are continuously extracted with pentane. The pentane is concentrated and washed with 72% sulfuric acid to remove carbazoles and similar type compounds. The components of the pentane extract are separated by gas chromatography, using a capillary column. The separated components are directly fed to a mass spectrometer and their spectra recorded.

A second four liters of water are extracted using an ion exchange column. The column is washed with sodium hydroxide and the effluent, after acidification, is extracted with chloroform followed by n-butanol. Each fraction is evaporated to dryness and the residues are methylated. The methylated residue is examined by gas chromatography, using a capillary column, and mass spectroscopy.

From the pentane extract, twenty-three compounds were identified from three different classes; n-alkanes, acid esters and polynuclear aromatic hydrocarbons. From the ion exchange extract, fifteen different acids were identified and ten additional compounds remain unidentified.

INTRODUCTION

This investigation was designed to develop and evaluate the application of gas chromatography-mass spectroscopy (GC-MS) as a qualitative analytical tool for the analysis of organic substances in fresh water. The classes of components selected were polynulear aromatic hydrocarbons (PNA) and organic acids. Our examination of PNA, using tandem gas chromatography-mass spectroscopy, was a collaborative study undertaken in this laboratory. The purpose of the study was to aid in the identification of those PNA which gave ambiguous results by a thin-layer chromatography-fluorescence method. The GC-MS system provided a means of identifying both fluorescent and non-fluorescent compounds and thus an evaluation of possible interferences (quenched or increased fluorescence) in the procedure.

In the examination of acids, a better understanding for the selective extraction of acids was sought using tandem GC-MS. The possible advantage of using an ion exchange technique for separation and concentration of organic acids in water was investigated as an alternative to the classical method. The classical method involves the partition of acids between an organic solvent and a basic solution. The selectivity of the ion exchange extraction was examined, qualitatively, for non-acidic compounds and for types of acids extracted. With a more thorough understanding of the nature of the acids contained in river water, it should be possible to develop quantitative methods which will be more selective and thus less susceptible to interferences.

The main reason for the high interest in PNA in the environment is because of their carcinogenic nature. The increased rate of lung cancer has focused attention on the

air we breathe. The reviews by Altshuller (1,2,3) on air analysis indicate the growing interest in methodology for PNA as evidenced by the increased number of papers published in the last several years. The acidic organic compounds in the atmosphere have received little attention. In fact, the reviews by Altshuller do not contain a single reference devoted to the analysis of carboxylic acids in the atmosphere.

The low solubility of PNA in water and/or the lack of a generally publicized health hazard, caused by the presence of these compounds in water, has not stimulated an extensive search for PNA in our water environment. Yet, it should be pointed out that Demisch (4) has found that the greater the carcinogenicity of the PNA toward the skin, the higher is its solubility in water.

The analysis of organic acids in water has received much attention. In part, this is due to the color and odor they give to water. The interest is divided into three groups; a) humic and fulvic acids, b) phenolic compounds and c) carboxylic acids. A review of the literature will show that the present interest in the carboxylic acid group is primarily for the low molecular weight acids with less than ten carbon atoms.

Separation and Concentration

The PNA in water are usually present in very low quantities and with many other types of compounds. Since the other compounds are present in larger amounts than the PNA, concentration and separation are essential to the analytical determination of PNA. Concentration and separation are important steps in the analysis of acidic compounds, but not to the same extent as for PNA. This is due to the higher concentration of acids in water and to the presence of the carboxyl group which makes separation more selective. The most widely

used methods of separating organics from water are the activated carbon adsorption method (CAM) and liquid-liquid extraction. It has been shown by Dornbush (5) and Robinson (6) that the CAM does not remove all the organics from the water. The efficiency of the CAM is dependent upon the pH of the water and the flow rate through the column. Robinson has connected two carbon adsorption filters in series. The pH of the water was increased before it entered the second filter. A larger amount of organic material was recovered with the second unit than with the first. This was attributed to the high pH of the water. Booth (7) and Lee (8) studied the optimization of flow rate and throughput volume for the CAM. Ward (9) has determined the influence of pH on the adsorption of certain acids by the CAM. At a pH of 3, approximately 50% of the acid in a 10^{-4} M. aqueous solution was adsorbed by the carbon. Coughlin (10) has shown that by chemically treating the carbon surface the adsorptive capacity can be greatly altered. Ettinger (11) has reported difficulties in obtaining complete removal of the organics from the carbon, due to the strong adsorptive property of the carbon. At best, using present technology, the CAM is capable of determining minimum concentrations.

Liquid-liquid extraction is the preferred method for concentrating small amounts of organic material from large volumes of water. Stanley (12) studied various organic solvents to determine their efficiency for extracting PNA from air particulates. Some of the solvents examined were pentane, benzene, cyclohexane, acetone, diethyl ether and methylene chloride. The study showed that no one solvent was 100% effective for all types of PNA. Several commercial solvents have been reported to contain PNA and several PNA were identified in all of the solvents examined (13). A careful

examination of the solvent used in the analysis of PNA is required. Khomenko (14) has studied several solvents, solvent combinations and different liquid extraction technique for their efficiency to separate and concentrate organic acids from water. The preferred method was to use an equal volume of butanol and to extract (3x5 mins.) at pH 2.

With both the CAM and the liquid-liquid extraction method, separation of the PNA and organic acids from other extractables is necessary. Several schemes for further concentrating and separating the PNA make use of partitioning between solvent pairs. Hoffman (15) partitioned PNA between cyclohexane and nitromethane, obtaining recoveries greater than 99%. A second scheme was based on the partition of PNA between an aliphatic solvent and dimethyl sulfoxide (16,17). Recoveries using this technique were lower than with the nitromethane method. Similar techniques have been used for acids. Braus (18) has used an aqueous sodium hydroxide solution to extract the acidic material from the organic solvent. Lead salts of saturated fatty acids can be separated from lead salts of unsaturated fatty acids by extraction with ether. The saturated fatty acid lead salts are more soluble in the aqueous phase (19).

For complex mixtures, column chromatography is not an unusual practice to effect additional separation and clean-up. The general procedure is to apply the sample to the head of the column. The column is first eluted with an aliphatic solvent to remove non-aromatic material. Next, the column is eluted with benzene and the aromatic fractions are recovered. Some loss usually results during the column procedure, however, due to irreversible adsorption or degradation. A similar procedure can be used in the analysis of acidic compounds. After the sample has been applied to the adsorbent, the column is eluted with chloroform to remove phenolic

compounds. Next, the column is eluted with n-butanol and the acid fractions are recovered.

Many adsorbents have been used in the separation of PNA. Klemm (21) has shown that on alumina PNA can be eluted in order of increasing ring number. Molecules with a large condensed ring system are more strongly bound, possibly involving π type complexation with active sites on the adsorbent. VanDuuren (22) has used an alumina column to chromatograph picrate derivatives of PNA.

Lijinsky (13) and Chakraborty (23) found silica gel to be highly effective in separating aliphatic compounds from the PNA. Silica gel is also the most widely used adsorbent in the separation of acidic organic compounds. Goncharova (24) used silica gel to fractionate a mixture of carboxylic acids by elution with varying concentrations of n-butanol in chloroform.

A magnesia/celite column has been used by several workers (13,16,17) to separate PNA from unwanted material. This adsorbent has two disadvantages. The adsorbent does not retain di- and tricyclic aromatic hydrocarbons (25) and exhibits a high affinity for the tetra- and pentacyclic aromatic hydrocarbons, resulting in low recoveries.

Florisil has been used by Bell (26) and Howard (16) to isolate PNA from aliphatic compounds. Recoveries from 75 to 95% for PNA were reported.

Howard (16) also used paper chromatographic procedures for the separation of PNA. A streak technique was used and several rechromatogrammings and extractions from the paper were required. The recoveries on the 19 PNA studied ranged from 62 to 96%. Grimmer (27) used paper chromatography for qualitative and quantitative determinations of PNA. Paper chromatography was used by Goncharova (24) to identify the

acids in the effluent of a chromatographic column. The identification was made on the basis of R_f values for the individual acids chromatogrammed on paper. Lamar (28) used paper chromatography in a study on organic acids. He reported that no distinct separation could be obtained when a mixture of many related acidic compounds were chromatogrammed.

Automatic liquid-liquid chromatographic methods have been used. Karr (29) analyzed PNA samples by liquid chromatography. Jentoft (30) separated PNA using high resolution liquid-liquid chromatography. Partition coefficients of some PNA between isooctane and several liquid substrates were determined; good separations of the PNA studied were obtained. However, rather long elution times were necessary. Goodman (31) determined trace amounts of organic acids by liquid chromatographic techniques. Cation exchange resins were used with automatic monitoring. More development in this area will be forthcoming as more commercial units are readily available.

Advantages inherent to thin layer chromatography (TLC) will result in the increasing use of this technique to resolve complex mixtures. The technique is simple, fairly rapid, inexpensive, versatile and sensitive. In addition, the high capacity exhibited by TLC makes this technique an attractive one.

Sawicki (32) has shown that TLC on alumina works well for the separation of fluorescent PNA. It has been shown by Berthold (33) that on alumina the R_f values for PNA were independent of the ring annulation type and showed a linear relation between adsorption and the ring content.

White (34) has reported that better resolution of PNA by TLC was obtained on silica gel than on alumina. However, the efficiency of extracting the PNA for quantitative work from both of these adsorbents is often poor. Kunte (34a)

found losses of from 4 to 17% resulted from quantitative TLC. The percent loss depended upon the compound examined. Oxidation and/or photodecomposition of PNA have been reported when using these adsorbents (13,15,35). Silica gel is the most often used adsorbent for chromatogramming acids. The handbook of Stahl (36) contains many examples of developing systems for the TLC of acids and acid esters on silica gel. Quantitative recoveries of acids and acid esters from silica gel are usually good.

Cellulose and cellulose acetate can be used to separate PNA by TLC. White (34) has reported that cellulose effectively separates PNA into groups according to ring structure. Cellulose acetate is superior in the separation of the individual 4-, 5- and 6-ring compounds. Quantitative recoveries of PNA from these two adsorbents are better than from either silica gel or alumina. Cellulose was used successfully by Baucher (37) to separate aliphatic dibasic and hydroxycarboxylic acids. He compared silica gel, silica gel-kieseguhr mixtures and cellulose for resolution of these acid groups. Cellulose, using an acid mobile phase, gave the best results.

Attempts have been made to modify the active sites of TLC adsorbents. Scholy (38) impregnated silica gel with polyoxyethylene glycol 1000 to improve separation for benz(a)pyrene. Short (39) reported that the separation of PNA was improved when the adsorbent was impregnated with an insoluble electron acceptor, such as pyromellitic dianhydride. Brightly colored spots fluorescing in the visible region allowed easy identification. Libickova (39) impregnated silica gel with a number of compounds having good electron-acceptor properties. The R_f values for the PNA decreased when compared with non-impregnated layers. The results varied depending upon the nature of the solvent system, the concentration of the impregnating agent added and the method of addition of the complex-

forming agent to the adsorbent. Similar techniques have been incorporated in the TLC separation of fatty acids. Bhatnager (40) used a mixture of urea and calium sulfate as adsorbent to TLC fatty acids. With this method, urea clathrate forms with the straight chain acids giving these acids lower R_f values. The method results in the separation of the acids into two classes, the straight chain acids and the branched or unsaturated acids. Coles (41) obtained poor separation using this technique to separate acids by TLC. Much wider use and success have been obtained by a technique called "reversed phase partition TLC". In "reversed phase partition TLC" the adsorbent is impregnated with a non-polar material to make it hydrophobic. This is accomplished by dipping the plate into a solution of the non-polar material in a volatile solvent and allowing the plate to dry. The mobile phase used to develop the plate is a polar solvent which is immiscible or slightly miscible with the non-polar material. Silicone has been widely used as a hydrophobic agent. Other hydrophobic agents include: undecane, tetradecane, squalane, polyethylene and paraffin. Several authors (42,43) have used this technique on a variety of acids and acid esters with considerable success. Several methods have been used in the detection and qualification of PNA in conjunction with previously reported separation techniques. In the column chromatographic methods, the effluent can be monitored continuously. Karr (29) and Jentoft (30) monitored the effluent using a flow cell in an ultraviolet spectrophotometer. Goodman (31) measured the change in the conductivity of the effluent from a cation exchange column for the analysis of acids. Shimomura (44) used a refractometer while Harlow (45) devised a method where the column effluent was titrated automically for emerging acids.

Lijinsky (13) and Howard (16) used paper chromatography to separate fluorescent bands of PNA. These bands were cut out

and eluted. The eluate was then examined by ultraviolet spectrophotometry. Color tests for PNA on paper have been reported using tetrachlorophthalic anhydride (46). Tetracyanoethylene and other reagents were used by Tarbell (47). Direct spectrofluorometric (48) and spectrophosphorimetric (49) analyses have been obtained on paper for both qualitative and quantitative information. Tiwari (50) converted fatty acids to their cobalt salts directly on the chromatographic paper. The salts were then reacted with ferricyanide to give green spots at a pH between 4 and 6. Bachur (51) used a nonspecific method for organic acids. The paper was sprayed with a pyridine solution followed by ultraviolet radiation at 253.7 nanometers. Halama (52) has evaluated direct photometry of fatty acids using paper chromatography. Many of the acid-base indicators could be used to visualize the organic acids on paper.

Reagents used to characterize PNA spots on thin layer plates include chloranil (39), pyromellitic dianhydride (38, 39) and 2,4,7-trinitrofluorenone (53). Dutta (54) used 2,7-dichlorofluorescein to visualize organic acids on thin layer plates. Sliwiok (55) evaluated acid-base indicators for their sensitivity to detect fatty acid. Most acid-base indicators can be used with a sensitivity of about 1 ug. Quantitative analysis by direct densitometry after visualization of fatty acid methyl esters was used by Osman (56). Densitometry was used by Kaufmann (57) on fatty acids and fatty acid methyl esters after charring.

Other workers (17,34) preferred to remove the spots from the plate and extract the PNA from the adsorbent with a suitable solvent. The solvent was examined by ultraviolet spectrophotometry.

Pfaff and Sawicki (49) measured the phosphorescence of PNA directly on thin layer plates. This technique offered

increased sensitivity and selectivity for a given number of compounds over other available methods, even fluorescence. Sawicki (58) and Keegan (59) used direct spectrophotofluormetric analysis of PNA on thin layer plates. A high degree of sensitivity for PNA was reported. The detection limit for most PNA was approximately 10 nanograms. Stahl and Jork (60) have reviewed several aspects of direct scanning with TLC.

Gas Chromatography

Gas chromatography (GC) offers the advantages of separation and detection without the disadvantage of additional sample handling between separation and detection. One need not elaborate on the advantages of this technique. The number of papers appearing in the literature on GC analysis speaks for itself.

A major problem in the GC analysis of pollutants is the very wide range of boiling points encountered in the analysis of a given type of compound. A second closely related problem is the increased number of isomers as the molecular weight increases. These problems must be resolved before this tool can be effectively used by the analyst. Based partly on these two problems, only a limited number of stationary phases has been used in the GC analysis of PNA. Many phases which could be used at the temperature required in PNA analysis do not provide the separation needed. The limiting features of most liquid phases are volatility and decomposition, resulting in high bleed of the stationary phase.

The bleed level is important for several reasons. A high bleed rate results in shortening the life of a column and produces changes in retention times with age and use of the column. The contamination of collected fractions also

results in complicating subsequent mass or optical spectra. A third problem is saturation of the detector. This could result in operation in the non-linear range of the detector and contamination of the detector normally results in reduced sensitivity. These factors greatly reduce the advantages offered by GC if left unattended.

The GC analysis of acids can be performed on either the free acids or on an ester derivative. For the long chain acids, the methyl ester is preferred because of the lower column temperature required. Stationary phases for ester derivatives can be of the nonpolar type, such as silicone greases, or of the polar type, such as polyester. With the nonpolar phases, the saturated acid esters emerge after the corresponding branched and unsaturated analogues. Using polar phases, the unsaturated acid esters emerge after their saturated analogues. The shorter chain acids can usually be successfully separated as the free acids. Since the free acids are less volatile than their corresponding esters, higher column temperatures are required. The chromatograms of the free acids tend to show considerable tailing and broad peaks unless specially prepared columns are used (61). James (62) added a small amount of stearic acid to a silicone oil. With this mixture as the liquid phase, a significant improvement in the peak shape was obtained for the free acids. Byars (63) modified carbowax 20M with 2-nitroterephthalic acids and obtained good symmetrical peaks with essentially no tailing.

Several other techniques are available to the analyst to help minimize the effects caused by the above-mentioned problems. The use of lightly loaded columns has several advantages. The amount of bleed is proportional to the flow rate and the amount of stationary phase. Thus, by using lightly-loaded columns the bleed is reduced. Lightly-loaded columns also allow elution far below the boiling point of the

solute. This has been enumerated by Karger and Cook (64). Frederick (65), using conventional supports and glass beads, attempted to find the lower limits of liquid load permissible. He investigated the effects of carrier gas velocity, column length and temperature on the efficiency of lightly-loaded columns. A method was devised for comparing the performance of columns. Abraham (66) used loads of 0.1-0.2% for several phases on glass beads in the GC analysis of PNA. It was found that for these columns the (HETP) occurred at a very low carrier gas flow rate and the HETP increased substantially with an increase in the flow rate of the carrier gas. These same phases were coated on celite-545 and were found to give good separation. The $(HETP)_{min.}$ occurred at a higher carrier gas flow rate and only a small loss in efficiency was obtained by increasing the carrier gas flow rate to higher values. At 225°C., naphthacene (b.p. 450°C) was eluted in 23 minutes on a column of 2% apiezon with celite-545 as support. Searl (67) used a 2%, SE-30 on chromosorb G (80/100 mesh) column and reported resolution as good as that achieved by capillary columns. Beeson (68) developed a new stationary phase for high temperature operation. With a 3% load of poly-m-phenoxylene good resolution for PNA was obtained. An operating temperature up to 380°C could be used with this phase. This phase eluted 1,2-benzpyrene and 1,3,5-triphenyl benzene in reverse order from that normally obtained with the silicone phases. Appleby (69) using lightly loaded columns of 3% polyethylene glycol or 3% embophase silicon oil obtained good separation of mono- and dibasic fatty acids as their n-propyl esters. Methyl ester derivatives were used by Lindemann (70) for the GC analysis of mono- and dibasic fatty acids. Clark (71) analyzed free fatty acids using a liquid phase of 5% carbowax and 5% isophthalic acid on embacel. Good resolution of the mono- and dibasic fatty acids was obtained.

Open Tubular Columns

Ettre (72) authored a monograph on open tubular columns. It contains information on coating, conditioning and storage for this type of column. A discussion of the "Golay equation" is given. The monograph provides an excellent summary of open tubular column technology. The influence of operating conditions on resolution was studied by Grushka (73). Parameters, such as liquid phase film thickness, column length and carrier gas flow rate were included in the study.

The efficiency of a column is measured by the number of theoretical plates. Packed columns usually contain around 2500-5000 theoretical plates. In capillary columns, the number of theoretical plates is around 50,000, although the number of plates can be as high as 200,000. With such high efficiency, capillary columns are well suited for the separation of complex mixtures. Elution and good resolution can be obtained in a reasonable time.

Liberti (74) used capillary columns in the GC determination of PNA. Elution of 22 PNA in less than 35 minutes was obtained on a capillary column of SE-30 operated at 200°C. Cantute (75) used a capillary column coated with SE-52. Thirty different PNA showed good separation. Averill (76) used a capillary column for the analysis of free fatty acids. Tailing was eliminated by the addition of dinonylnaphthalene acid. The separation of the methyl ester of fatty acids using a capillary column has also been performed (77). Good separation was obtained; however, the efficiency did not appear to be significantly better than with a good 1/8" packed column.

Programmed Temperature GC

Harris and Habgood (78) published a comprehensive treatment on the theoretical and practical aspects of programmed

temperature gas chromatography (PTGC). Schulz (79) studied the efficiency of capillary columns under temperature programming.

In PTGC, the column temperature is constantly rising. The vapor pressure of the stationary phase increases with an increase in column temperature. Since carrier gas is constantly flowing across the phase, bleed occurs continuously. The rate of bleed will increase during programming, thus causing base line drift. With the sample peaks superimposed on the drift, peak analysis becomes very difficult. Levy (80) has suggested the addition of a short column containing a liquid phase of very low volatility at the end of the analytical column. This column traps the bleeding stationary phase from the analytical column and does not effect the overall efficiency of the column. It was found to be superior to the dual column technique and allowed working at high sensitivity.

Lijinsky (81) used PTGC to resolve and identify PNA in high temperature tars. A temperature program from 100 to 200°C was used with a nonpolar stationary phase. Component peaks were symmetrical and resolution at both the high and low boiling end was better than that obtained from an isothermal run. Sensitivity at the high end was better due to sharper peaks and analysis time was greatly reduced from an isothermal run. Temperature programming was used by Maruyama (82) to separate a mixture of free fatty acids. Symmetrical peaks and complete separation were reported for C_5 to C_{22} fatty acids at column temperatures not exceeding 200°C. Smith (83) used PTGC to separate esters of fatty acids. Separation was obtained according to the chain length of the acids. A programming range of 100 to 210°C at 3°C per minute was used.

Gas Solid Chromatography (GSC)

Only partial success in resolving the important benzpyrene fraction has been achieved with organic liquid phases. For this reason GSC has been attempted in an effort to improve separations. An added feature of this technique is the thermal stability of the stationary phase. This permits higher operating temperatures with little or no column bleed.

Chortyh (84) made a limited study using alkali and akaline earth chlorides as the stationary phase in the analysis of PNA. A column with 20% LiCl on chromosorb-P was used. With temperature programming, the benzpyrene isomers were resolved. Gump (85) compared a number of alkali and alkaline earth salts on various supports. It was shown that the choice of the solid support material has an effect on the performance of the salt packing. It was also shown that the firing temperature affected the performance of the column and it was not predictable from one salt to another. None of the salt columns was capable of giving baseline separation of the benzpyrene fraction. The LiCl and the CsCl column showed the most promise.

Gump explained the separation as resulting from weak bonding forces between the salt and the PNA. The less symmetrical ring compounds appeared to be adsorbed more than the symmetrical compounds. Sawyer (86) showed the interaction to result from a combination of specific and nonspecific contributions. The specific contribution included those due to the π character of the solute (PNA) molecule and depended upon the nature of the salt and the adsorbent.

Altenau (87) used copper-amine complexes as stationary phases. A 10% $Cu(Bipy)_2(NO_3)_2$ (copper bipyridyl nitrate) on chromosorb W column was used to separate some aromatic hydrocarbons. Column temperatures up to 225°C were studied without

any evidence of bleeding or decomposition. Aromatic hydrocarbons boiling up to 150°C above the column temperature gave symmetrical peaks. Sample sizes of from 0.1 to 5 ug could be used.

Detection

A variety of detectors has been used in the GC analysis of PNA. The greatest interest has centered upon the flame ionization detector (FID) and various types of electron capture detectors (ECD). These detectors, because of their high sensitivity, provide the response needed for the analysis of trace components.

Pinchi (88) has reported that while the FID was extremely sensitive to aromatic hydrocarbons, it was found that the response was unusually poor for some compounds. Liberti (74) and Cantuti (75) have reported data on the weight response of some PNA using a FID. The response of the FID to the various PNA showed no simple relationship between the number of carbon atoms and the response of the detector. For example, two aromatic compounds having the same molecular weight (mol wt 202) showed a difference in response of 1.4 (pyrene over fluoranthene). The relative response (methyl palmitate = 100) of the FID for fatty acid methyl esters was determined by Ettre (89) using methyl palmitate as an internal standard. With the FID the relative peak area can be related to the weight concentration. The relative responses for equal weights of fatty acid methyl esters are equivalent when determined on a FID. The FID response for saturated and unsaturated fatty acid esters were equivalent, within experimental error, on a weight concentration basis.

The electron capture detector (ECD) offers several advantages over the FID. Guvernator (90) has studied under

which conditions the most useful results could be obtained for the detection and identification of PNA using the ECD. Various factors affecting the detector's sensitivity were included. A wide variety of electron capture detectors are available. The tritrium source ECD has the disadvantage of a maximum recommended operating temperature of 225°C. At this temperature, contamination of the source due to condensation of the PNA, soon results in lower sensitivity. The Ni^{63} detector can be used up to 355°C. This higher operating temperature minimizes contamination due to condensation. The helium glow discharge ECD used by Davis (91) in the analysis of PNA was capable of operating with high sensitivity up to 400°C. Kawahara (92) used an ECD in the analysis of organic acids. The pentafluorobenzyl ester derivatives of the organic acids were required to convert the acids to strong electron absorbing molecules. Unknown substances which did not react with the derivatizing agent were not detected in the GC measurement.

As with the FID, the ECD has shown a difference in weight response among various PNA (75). The response varies somewhat, depending upon the conditions used. Guvernator (90) reported that the more unsymmetrical the molecule, the greater the response of the ECD for the PNA. It was also reported that with the ECD, two PNA could reverse their relative response at different detector voltages. The sensitivity of the ECD is on the order of 1 to 10 nanograms.

Several attempts (93,94) have been made to link the GC to a spectrophotofluorometer. The fluorescent detector has a high sensitivity for PNA. Its response can be made selective by the proper choice of excitation and emission wavelengths, though the level of detection varies from compound to compound. These papers describe a method for suitably linking the two instruments.

Qualitative Analysis by GC

Attempts have been made to qualitatively identify PNA using relative retention values obtained by GC. If one plots the logarithm of the retention time of a homologous series of organic compounds versus the carbon number, a straight line is obtained (95). This technique was used by Solo (96) to identify about 60 alkylated derivatives of phenanthrene. Abraham (97) used relative retention times for the qualitative analysis of PNA. He reported that values obtained from various sources do not always agree and that some degree of scatter of points about the line was obtained. This can be related to the fact that the PNA studied do not form a true homologous series. Sawicki (98) reported that to unequivocally identify large conjugated molecules more than the R_f value or the retention time was required, but that such qualitative studies were a necessary preliminary step to any more thorough investigation of the quantitative composition of our environment.

Miwa (99) used a system called "equivalent chain length" (ECL) for the GC characterization of fatty acid methyl esters. The ECL system is independent of operating conditions such as column temperature, carrier gas flow rate and column dimensions. The ECL system involves a semilogarithmic plot of the retention time (log scale) of several normal, saturated fatty acid methyl esters versus the number of carbon atoms in the acid. The carbon number of subsequent acids chromatogrammed under the same GC conditions was read from the curve using the observed retention time. Only the slope of the curve varied with GC conditions; the ECL values remained constant. Lamar (100) successfully identified a number of fatty acids extracted from water using the ECL method. Identification was made using a polar and a non-polar column to obtain retention values. Iverson (101) evaluated isothermal semilogarithmic plots, the

retention time ratio method, programmed temperature semilogarithmic plots and the variation of retention time with temperature method for the identification of fatty acid esters separated by GC. It was concluded that unequivocal proof of the presence or absence of a particular acid present in trace amounts was difficult using only GC methods.

Gas Chromatography-Mass Spectroscopy (GC-MS)

A number of comprehensive reviews dealing with the coupling of a gas chromatograph to a mass spectrometer are available (102-105). Rees (106) reviewed the many types of interfacial systems available and the advantages and disadvantages of the most prominent types of systems were given. Factors important to the proper selection of a particular type of separator were also given.

The GC and MS have two compatible features. First, both instruments are capable of utilizing less than microgram amounts of material. Secondly, the analysis is performed in the vapor state. However, an inherent incompatibility does exist. The outlet of the GC is usually operated at ambient pressure. Grayson (107) and Walsh (108) have reported the results of their studies on the selection of the optimum pressure range in the ion source. It was determined, that for the mass spectrometer to operate efficiently, pressures less than 10^{-5} Torr were required. Two approaches have been used to minimize the pressure in the ion source. The first method makes use of the low flow rate characteristic of capillary columns. The second method utilizes a molecular separator to remove some of the carrier gas, thus concentrating the eluent. Using capillary columns, flow rates on the order of 0.2 to 2.0 ml per min are required. All of the effluent goes to the mass spectrometer. The minimum sample required is about 10^{-10} gms

per sec. Capillary columns with diameters of 0.02 or 0.03 in. i.d. require higher flow rates and therefore part of the effluent must be split or the use of a separator must be incorporated. The separator allows higher flow rates to be used and as an enriching device, it permits a larger percentage of the total effluent to be introduced into the mass spectrometer, thus providing good spectra with discernible peaks of adequate intensity. This is accomplished without an increase in background pressure to levels which could result in mass discrimination, peak broadening and a deterioration of resolution.

Certain problems can result from the use of molecular separators. The partial loss of sample material is unavoidable. The loss can be expressed in terms of the efficiency of the separator. The term efficiency is used to describe that fraction of the injected GC sample which reaches the MS ion source after the enrichment process. The efficiency of all available separators is less than 100%. For example, Grayson (107) found that the efficiency of the fritted glass separator was between 15 to 65%. The loss of sample is due to effusion through the frits and from adsorption, as noted in studies where trace quantities of alcohols and other polar compounds were analyzed (109). Silanization of the separator did not completely eliminate the sorption of small quantities of sample, but did reduce the amount of material needed for detection.

Levy (110) investigated the effects of dead volume and unswept volumes in the vacuum lines of the MS inlet system connected to the GC. It was shown that dead volume has little or no affect on the resolution of a chromatogram as determined by the total ion monitor. Stalberg-Stenhagen (105) reported no problem due to a memory effect, provided that the connecting line between the GC and the ion source was kept at a uniform

temperature and that no dead volume was present in tubes or valves that carry gas at higher pressure.

Bleed becomes an important factor in temperature programming in conjunction with GC-MS. Dual column systems used to compensate for column bleed cannot be used in a GC-MS on-line system. Since all GC stationary phases bleed to some extent, consideration must be given to the type of phase and operating temperature used for GC. The sensitivity of the GC-MS system is limited by the amount of column bleed (111). An additional problem results if bleed of the liquid phase tends to mask the spectral pattern of the sample. Levy (112) minimized bleed to the MS ion source by adsorbing it onto an attached short column of high thermal stability at the end of the GC column.

For samples less than a microgram and for tandem GC-MS, fairly rapid magnetic scan rates are required. The rate depends upon the mass resolution and the time constant of the amplifier and the recorder. In order to obtain a characteristic fragmentation pattern of relative ion abundance, the amount of sample in the ion source must be constant during the scanning. As the GC peak elutes from the column, the concentration is changing with time. If the time required by the MS to scan an eluting peak is equivalent to the peak's width, the intensity of the ions at the start and the end of the mass spectrum will be greatly diminished due to the lower concentration in these portions of the GC peak. The intensity of the ions will be enhanced, due to the greater concentration near the center of the eluting GC peak. To minimize this distortion, it is common practice to scan rapidly close to the top of the GC peak. The best mass spectrum is obtained by using the slowest possible scan rate consistent with the elution rate of the GC peaks.

EXPERIMENTAL

Gas Chromatography

The gas chromatographic work in this study was done on a Perkin Elmer, model 881, gas chromatograph equipped with a temperature programming unit. Twelve linear programming rates were available from 0.5°C per min. to 48°C per min. The model 881 contains dual injector ports and dual flame ionization detectors. The instrument can be operated in either the dual column or single column mode.

Independent heating controls for the injector block, column oven and detector are standard equipment. Since the detectors were located within the column's oven, the detectors maximum and minimum temperatures were dependent upon the column's operating temperature. With high temperature operation this was no problem. Near ambient temperatures, maintaining both the oven and detectors at a given temperature became difficult due to convection heating from the heated injector block.

Glass injector ports were used throughout this study. Pyrolytic decomposition was thus minimized in the injector block unit.

Optimization of the Flame Ionization Detector

The hydrogen and air settings for the flame ionization detector were determined using a 2n factorial design experiment. The "n" represents the number of variable to be studied and "2" represents the number of settings to be used for each of the variables. "High" and "low" settings were used. A "center" point was also used. The two variables of interest

were the flow of hydrogen and the flow of air required for maximum response. Fluoranthene was chosen as the standard for this experiment. The GC conditions were chosen to give the shortest possible retention time for fluoranthene while completely resolving it from the solvent peak. The carrier gas flow rate was fixed at 6 ml per minute.

The results are summarized in Tables I and II. Table I contains the design matrix. The zeros represent the "center" point position. The "center" point is helpful in determining the response surface at the center of the design and makes the design more sensitive by allowing the use of quadratic terms. All data points were obtained in random fashion.

The design matrix of Table I consists of four columns. Column A and column B represent the variables of interest, the hydrogen and air flows, respectively. Column AB represents the interaction effect. The interaction is a measure of the non-additivity of the main effects. It was assumed that only two-factor interactions were involved and that the carrier gas flow rate did not interact with variable A and B. This was not an unwarranted assumption because three and higher order interactions are considered physically unrealistic. The Lack of Fit column compares the center point of the design matrix with the peripheral points.

A common sense approach which employs changing one variable at a time was used to get near maximum response on the flame ionization detector. The values for the hydrogen and air flow obtained with this approach were used as the center point in the design matrix. With these values as the center point we should expect the detector to have near maximum response at these settings. To state it differently, the reaction surface generated by the flame response should have its line of steepest decent starting at the center point.

To help in the analysis of the collected data, an

TABLE I

Design Matrix for the Optimization of the GC Flame Ionization Detector

Variable A Hydrogen	Variable B Air	Interaction AB	Lack of Fit	Detector Response
-1	-1	+1	+1	9.5
-1	-1	+1	+1	7.0
-1	-1	+1	+1	8.0
+1	-1	-1	+1	15.5
+1	-1	-1	+1	15.0
+1	-1	-1	+1	13.0
-1	+1	-1	+1	12.0
-1	+1	-1	+1	14.0
-1	+1	-1	+1	13.0
+1	+1	+1	+1	18.0
+1	+1	+1	+1	19.0
+1	+1	+1	+1	20.0
0	0	0	-4	28.0
0	0	0	-4	30.0
0	0	0	-4	29.0

Analysis of Variance table was set up (Table II). The Total Sum of Squares, y^2, is a measure of the total randomness of the system. The Total Degrees of Freedom is a measure of the number of data points collected. The Correction Factor, $(y)^2/n$, corrects the experimental values to a mean of zero; "n" equals the total number of experimental points. The degree of freedom in this row is one. The difference between the total sum of squares and the correction factor is the variation caused by changes in the experimental conditions and random error. The Error Sum of Squares was obtained by running multiple measurements; it is a measure of the variation in the measurements for a given set of conditions.

By comparing the variance of one variable with the random error, one can determine the significance of a given effect. This is performed by comparing the Mean Square value (the sum of squares divided by the degrees of freedom) for the variable to the error mean square. The quotient calculated is called the F ratio. For a variable to be significant, its F ratio must be larger than the F value obtained from standard tables for the same degrees of freedom.

With completion of the analysis of variance table, an interpretation of the data can be made. In Table II, variable A shows a F value of 89 as compared to 4.96 at the 95% confidence level and 10.04 at the 99% confidence level. The result implies that there is more than a 99% probability that a change in hydrogen flow rate results in a significant change in the flame's response. Likewise, a change in the air flow would also result in a change in the flame's response at the 99% confidence level. This would not be as critical for the air flow because the difference in the tabular F value is smaller. The interaction of variables A and B is shown to be not significant. For the Lack of Fit measurement, the F value was highly

TABLE II

Analysis of Variance Table for Optimization of Flame Detector

	Effects	Sum of Squares	Degrees of Freedom	Mean Squares	F Ratio
Total		4956.60	15		
Correction Factor		4200.60	1		
Variable A	-6.17	114.08	1	114.08	89
Variable B	-4.33	65.33	1	65.33	51
Interaction AB	-0.17	0.08	1		N.S.
Lack of Fit		564.24	1	564.24	441
Error		12.80	10	1.28	

F (1,10) at .95 = 4.96
.99 = 10.04

N.S., Not Significant

significant. This was predicted because the design had been set up to give a non-planar response surface; that is, near maximum response was expected for the center point.

The Effect of Variables A and B, with their positive signs, implies that higher flow rates for both hydrogen and air would result in a higher flame response. A common sense approach was used to make the final adjustments of the flow rates, making use of the information from the Analysis of Variance table. The GC conditions are reported in Table III.

Mass Spectrometer

The mass spectrometer used in this study was an Hitachi RMU-6E instrument. The entrance slit to the analyzer was set at a fixed slit width of 0.2 mm. A variable exit slit was used. Under normal operation, the exit slit was set a 0.4 mm. With this slit arrangement, a resolution of 700 (10% valley definition) was obtained when calculated using the mercury peak at m/e 200.

The instrument was equipped with a Honeywell, Model 1706, Oscillographic Visicorder. The visicorder was a direct-reading oscillograph designed to record four channels simultaneously. The recording frequency range was from DC to 5000 cycles, with a maximum writing speed of over 30,000 ins. per sec. Eight paper speeds were available, 6.0 mm. to 800 mm. per sec.

Using the visicorder, the reading of a mass spectrum from 0 to 300 mass units was performed in 3.0 seconds. A narrower mass range was used when the molecular weight of the compounds being examined was known. The scan speed and paper speed remained unchanged. This rate allowed taking several scans during the emergence of a single gas chromatographic peak.

The complete operating conditions for the mass spectrometer

TABLE III

GC Operating Conditions for PNA Analysis

COLUMN	Length	50 feet	Dia.	0.02 in. i.d.
	Coating	SE-52	Conc.	Wall coated open tubular
	Support	none	Mesh	none
TEMPERATURE	Column: Init.	150°C	Final	190°C
	Rate	4°C/min	Det.	225°C, Inj 225°C
CARRIER GAS	Helium		Rate	6 ml/min
	Pressure	Inlet - 20 psig	Outlet	Sub-ambient
	Hydrogen	13.5 psig	Air	40 psig
DETECTOR	FID			
	Sens	XI	Recorder range	1 mv.
SAMPLE			Size	1-3 µl
	Solvent	Benzene	Concentration	Variable

are shown in Table IV.

Connecting the Gas Chromatograph to the Mass Spectrometer

The gas chromatograph was connected to the mass spectrometer by means of a 0.01 in o.d. x 4.5-ft stainless steel capillary tubing. The capillary was enclosed in a 0.25 in o.d x 4-ft aluminum tubing for added strength and protection. Two thermocouples were attached to the outside of the aluminum tubing. The thermocouples were spaced one foot from each end of the aluminum tubing. The temperature of the line was read on a Perkin Elmer Temperature Controller by means of the two thermocouples. The aluminum tubing and thermocouples were wrapped with heating tape. The "Heat-by-the-Yard" tape, 0.5 in. wide x 8-ft. long, was purchased from the Fisher Scientific Company. The temperature of the tape was controlled by a variable autotransformer.

The capillary line was connected to the exit port of the receiving block in the gas chromatograph. The connection was made using a flow restrictor. Swagelok fittings and a Swagelok reducing union connected the restrictor to the capillary tubing. The restrictor was shortened, approximately two inches, to allow the connection to be made within the oven compartment of the chromatograph.

The mass spectrometer end of the capillary tubing was attached to a heated micro-capillary valve. The valve permitted separate operation of each instrument. The valve and capillary line were connected by means of 1/16 in. Swagelok fittings within the heated inlet box of the mass spectrometer. The valve was connected to the inlet side of an all-glass Watson-Biemann Separator by means of a 1/16 in. o.d. stainless steel capillary through a Kovar, metal to glass seal.

A block diagram of the complete system is shown in Figure 1.

TABLE IV

Mass Spectrometer Conditions

Connecting Line Temp. 225°C — Setting 70

Inlet Box Temp. 275°C — Setting 95

Ion Monitor Plate varies — Sensitivity X1, X3

Recorder 1 mv

$\frac{m}{e}$ 450 — Accel. voltage

Repeller I 6 V — Grid I -10 V

Repeller II 7 V

Pressure* 4×10^{-6} Torrs

Emission Regular

Filament 3.4 A — Chamber 20 eV

Total 50 A — Target 80 V

Target 20 A — Grid -20 V

Repel I 6 V

Repel II 7 V

Electron multiplier

EM 1500 voltage — Sens X10

G Heater 0.7 A, ---- Ch. Heater 3.0 A, Temp. 250°C

Visicorder paper speed 200 mm/sec

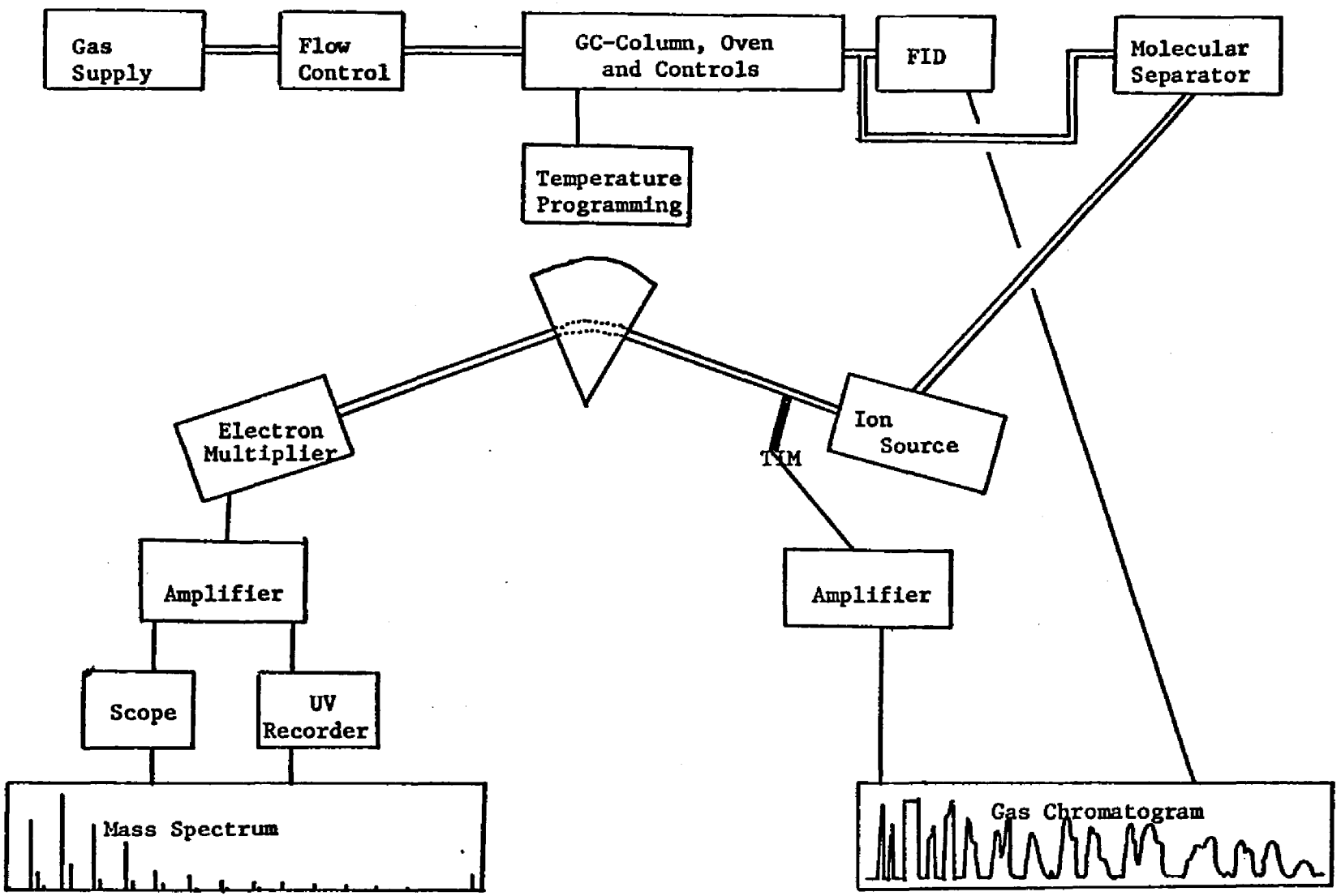
Coil Current: Start 0^{mA} — End 250 mA

Scan speed 12 — Time 3.0 sec.

*with GC line open

Figure No. 1

Block Diagram of the GC-MS System



Smallest Detectable Concentrations for the GC

The smallest detectable concentration was determined for fluoranthene, benz(b)fluorene, benz(a)anthracene and benz(a)pyrene. A series of standard solutions containing the four components as a mixture were made. Each solution contained decreasing amounts of the compounds. For each injection, a constant volume of the standard solution was used. The GC conditions used are shown in Table III. These conditions are identical to those used for the GC-MS runs, except that the connecting line between the two instruments was in the "close" position. The smallest detectable amount was defined as that amount which gives a GC signal twice that of the noise level. The results are shown in Table V.

Fraction Going to the MS System

Fluoranthene was used as the standard to determine the fraction of the GC effluent going to the MS. The line to the mass spectrometer was closed and GC conditions were as reported in Table III. An amount of Fluoranthene was injected into the GC sufficient to produce a detector response of around 80 to 90% full scale. Three identical injections were made. The line to the mass spectrometer was then opened. The conditions for the mass spectrometer and connecting line were shown in Table IV. Three additional injections were made. The chromatograms were recorded as in the previous runs. The detector's response was lower with the mass spectrometer line opened. The difference in detector response represented the amount of fluoranthene going into the connecting line and to the mass spectrometer. The result indicated that under the experimental conditions used, 42% of the fluoranthene entered the connecting line. Of the 42% going to the mass spectrometer, part will be lost in the molecular separator. Therefore, not all of this amount

TABLE V

Smallest Detectable Concentration Using FID

Compound	M.W.	R_t	Concentration
Fluoranthene	202	2.7 mins.	0.14
Benz(b)fluorene	216	3.5 mins.	0.31
Benz(a)anthracene	228	5.4 mins.	0.74
Benz(a)pyrene	252	14.5 mins.	2.00

One microliter of sample injected

will reach the ionization chamber. No attempt was made to determine the amount lost in the separator unit. Also, no attempt was made to determine the amount entering the connecting line for other compounds. The amount will vary and depend greatly upon the carrier gas flow rate. The amount lost in the molecular separator will decrease with increasing molecular weight, since the rate of diffusion through the glass decreases with increasing molecular weight.

Smallest Detectable Concentration for MS Analysis

The standard solutions prepared for the determination of the detection limits were also used to determine the detection limit for the mass spectrometer. A sample size sufficient to give a GC peak was used at all times. The mass spectrum of the GC peak was taken at the peak's maximum. Several GC-MS runs were made. The concentration of the sample was decreased for each injection. By plotting intensity of parent ion vs concentration and calculating for approximately 42% of the fraction goes to the MS, it was possible to approximate concentration needed to obtain identifying spectra for the four compounds investigated. The results are shown in Table VI.

The GC-MS conditions used are presented in Tables III and IV.

Standard Solution of n-Alkanes

A standard solution of n-alkanes was used to check out the overall operating conditions of the GC-MS system. The alkane solution consisted of five even-numbered carbon atom alkanes, C_{10} through C_{18}. The chromatogram for the GC was recorded using the flame ionization detector. The chromatogram at the mass spectrometer was recorded using the Total Ion Monitor (TIM). The TIM consists of a monitor head and an

TABLE VI

Smallest Detectable Concentration Using MS

Compound	M.W.	Concentration* ug
Fluoranthene	202	0.07
Benz(b)fluorene	216	0.15
Benz(a)anthracene	228	0.37
Benz(a)pyrene	252	1.00

*Based on approximately 42% of injected concentration going to the mass spectrometer.

One microliter of sample injected.

electrometer amplifier. The head is a gold foil Faraday collector placed at the entrance to the magnetic sector. The gold foil is externally retractable from the ion bean and can be adjusted to collect part of the ion beam. The ion current is fed to a meter (10-12 A) or can be fed to a millivolt recorder. The TIM allows continuous recording of the ionized gas as it leaves the ionization chamber of the mass spectrometer. The TIM recording is very similar to that obtained by the FID of the GC. The separation efficiency as measured with the TIM is less than for the FID. If the system was operating ideally, the chromatograms recorded with the TIM and the FID would be identical. If, for example, a "cold spot" existed in the connecting line the TIM chromatogram would differ from the FID chromatogram. The difference would show up in "tailing" of the TIM recording. This was not observed and symmetrical peaks were obtained, using the previously described system.

The standard alkane solution was also used to determine the loss in separation efficiency due to the connecting line between the GC and MS. Chromatograms were recorded using both the FID and the TIM. The number of theoretical plates for each compound was determined from the chromatogram obtained from each detector. The results are shown in Table VII.

The number of theortical plates, N, was calculated using the following formula, $N = 16\ (X/Y)^2$. Where X equals the distance from injection to the peak maximum and Y equals the width of the peak at the base line.

The difference in the number of theoretical plates indicates a loss of resolution in the connecting line. This could result from dead volume or unswept volume. An unswept volume existed in the GC-MS system used in this study. The GC inlet to the MS was made on the analyzer's side of the molecular leak. The molecular leak was installed into the

TABLE VII

Comparison of Efficiency with the
GC and GC-MS Systems

	Number of Theoretical Plates	
Compounds	FID	TIM
n-Decane	1024	483
n-Dodecane	1600	739
n-Tetradecane	3136	1506
n-Hexadecane	11237	5536
n-Octadecane	23347	11491

system by means of a ring seal. To prevent damaging the ring seal, the GC inlet was made about three inches from the molecular leak. This created an unswept volume of about 7 ml and may account for part of the loss in efficiency. An additional factor to be considered was the distance between the two detectors. This distance amounts to approximately 10 feet. It is believed that these two factors contributed greatly to the lower efficiency calculated for the total system up to and including the TIM. Although the peaks were symmetrical and minimum tailing occurred, the available data does not completely rule out the existence of "cold spots".

Preparation of Water Samples for PNA Analysis

The water samples were collected using five liter Nalgene Polyethylene Carboys and immediately transported to the laboratory. Upon arrival, the water to be analyzed was transferred to extraction vessels. Two, 4.5 liter continous liquid-liquid extractors were used. Standard tapered glass joints allowed fixing a water cooled condenser to the top of the extractor and a solvent pot was connected by means of a side arm. The extractor was designed for use with organic solvents lighter than water, thus the side arm was attached just above the 4.5 liter capacity of the extractor. The solvent was evaporated from the extracting solvent reservoir by means of a variac-controlled heating mantle. The vapors were condensed in the water-cooled condenser and the solvent droplets fell into a thistle tube placed directly below the condenser. The thistle tube extended down into the water and the lower end of the tube was about one inch above the flat base of the extractor. The lower end of the thistle tube contained a small fritted glass disk, through which the organic solvent was forced as a steady stream of very small bubbles.

A magnetic stirring bar was used to help speed up mixing and shorten the time required for extraction. The water was continuously extracted using n-pentane as the extracting solvent. The pentane was adjusted to a given volume and the fluorescent intensity was recorded periodically. When the fluorescent intensity remained constant, the extraction was considered complete. This usually required about 24 hours. The volume of the pentane was reduced to 25 ml and quantitatively transferred to a separatory funnel. The pentane was extracted with 72% sulfuric acid to remove basic compounds, particularly heterocyclics. After the acid wash, the pentane layer was dried, concentrated and the volume adjusted. Part of the sample was taken for TLC-Fluorescence analysis and the remaining portion was used for GC-MS analysis.

Sample Preparation for GC-MS Analysis

The pentane sample was transferred to a 3 ml tapered centrifuge tube. The solvent was evaporated to dryness using a stream of dried-filtered nitrogen. A very low flow of nitrogen was used to prevent the pentane from creeping up the side of the container. The residue was dissolved in 25 microliters of redistilled benzene. One to three microliters of the benzene solution were injected into the GC. Conditions for the GC are shown in Table III, while the MS conditions are given in Table IV.

Several factors should be noted. First, all spectra were recorded at 20 eV. Second, the filament, target and total current of the emission regulator panel were adjusted to recommended values. Good sensitivity was obtained at these settings. Lastly, the ion monitor plate was adjusted to intercept about 20% of the ion beam. This adjustment was made by setting the magnetic field strength to sit on a peak maximum.

The total ion monitor plate was then inserted into the ion beam until the peak's intensity was reduced by 1/5. At this setting, the chromatograms were continuously recorded. The mass spectra were reduced approximately 20% in intensity. This procedure allowed a first-hand look at the compound as it entered the mass spectrometer and indicated the optimum time to obtain a mass spectrum.

Preparation of Ion Exchange Column

Ion exchange was used to remove acidic compounds from natural river water. Columns were prepared using Amberlite IRA-458. This ion exchanger has a hydrophilic resin structure. It exhibits less adsorption of organic species than do other resin matrices which have a more hydrophobic structure. The resin has a high capacity; rapid removal of anions during the regenerating cycle was common.

A 50 ml laboratory burette was used as the column. The resin was held in place by a glass wool plug at both the top and bottom of the resin bed. The column was packed using a wet method. The burette was partly filled with water. The resin was then poured into the burette from an aqueous slurry. A slow drain from the burette was allowed during the addition of the resin. To ensure a uniform packing and to remove air bubbles from the bed, the column was backwashed.. Care was taken to avoid stepwise settling of the resin while backwashing.

The resin was used in the chloride form. A bed volume of 10 cm. was washed with 25 ml of a 10% NaCl solution to ensure complete conversion to the chloride form and to prevent elution of extraneous resin material during the regenerating step with a NaCl solution. Following this, the resin was washed with 100 ml. of distilled water to remove excess NaCl from the system. Once the column was free of NaCl, the column

was ready to use.

Preparation of Water Samples for Acid Analysis

The water samples were collected using five liter Nalgene Polyethylene Carboys and immediately transported to the laboratory. The water was filtered through a double layer of S & S No. 595 fine filter paper. The pH of the filtered water was adjusted to a value of 2. The sample was transferred to a clean glass container. The sample was left undisturbed for two to five hours. After this time, the water sample was again filtered through a double layer of S & S No. 595 fine filter paper. A dark brown material was collected on the paper for all water samples examined using this technique.

The pH of the freshly filtered sample was checked and adjusted to a value of 2 if needed. The sample was again left undisturbed for two additional hours, and then refiltered. No additional material was collected on the filter paper. The sample was now ready to be passed through the ion exchange column.

Three and one half liters of the acidified water sample were transferred to a constant feed reservoir attached to the ion exchange column. The flow rate was adjusted to 8-10 ml per min. A leveling device was used to prevent the column from going dry and the column was allowed to run unattended overnight.

The white resin beads gradually changed to a light yellow color. As the volume of water passed through the column increased, the color changed to a light brown. Near the end of the run, the top of the column was brown and the entire column contained colored material.

After passing 3.5 liters of river water through the resin, the column was washed with 100 mls of distilled water

(pH adjusted to 7). The column was eluted with 1N NaOH and 100 mls of the effluent were collected. Some colored material was eluted from the column. A second elution using 10% NaCl was performed and 50 mls of this effluent were collected. Additional colored material was removed from the resin. No attempt was made to try and elute all of the colored material from the resin.

The effluent from the NaOH washing was reduced to approximately 10 mls by means of a vacuum rotary evaporator. A heated water bath maintained at 55°C was used to hasten the process. The remainining solution was acidified with concentrated HCl to a pH of 2 and transferred to a separatory funnel. The acidified sample was extracted once with 10 mls of $CHCl_3$ and four times with 5 mls of $CHCl_3$. The $CHCl_3$ was dried over Na_2SO_4 and evaporated to near dryness by means of a rotary evaporator. The remaining solvent was driven off with a fine stream of dried-filtered nitrogen. This sample was saved for methylation and GC-MS analysis.

It was expected, under the conditions of the extraction, that most of the acidic compounds should be extracted by chloroform. Because the extracted aqueous phase still possessed a slight yellow color, a second extraction was performed to determine the types of acids not extracted by chloroform and the possible cause of the yellow color. The more polar solvent, n-butanol, was used for the second extraction. The butanol was saturated with 2N HCl and the aqueous phase was extracted three times with 8 mls of the HCl butanol solvent. The butanol extract was evaporated to dryness by means of a vacuum rotary evaporator. The organic material was taken up in acetone to separate it from the salt present. The acetone was removed with a stream of dried-filtered nitrogen and the residue saved for methylation and GC-MS analysis.

Methylation of Acids

Methylation of the chloroform and n-butanol extracts was performed by means of a 10% BCl_3MeOH solution. To each extract was added 5 mls of the methylating solution. The solution was brought to a boil and allowed to remain at room temperature in a closed container overnight. The samples were transferred to separatory funnels and 100 mls of a slightly basic (pH 7.5-8.0) aqueous solution was added. The samples were extracted five times with 6 mls $CHCl_3$. The basic solution served to remove any unreacted acid which might interfer with the GC separation or mass spectra of the methylated derivative. In both the chloroform and n-butanol extracts, all of the yellow color was extracted into the $CHCl_3$ layer after methylation. The $CHCl_3$ was dried over Na_2SO_4 and reduced to a volume of approximately 1 ml. The samples were transferred to 1 dram vials by means of transfer pipettes. The two extracts were examined by GC-MS. The GC conditions for the methyl ester of the acids are given in Table VIII and the MS conditions are given in Table IV.

Location of Water Collection Sites

All water samples analyzed were taken from rivers in the State of New Hampshire, primarily near the University of New Hampshire in Durham, New Hampshire. The specific locations are as follows:

Sample No. 1 was collected April 21, 1968 from the Cocheco River. The collection was made near the bridge on County Farm Road just above the County Farm.
Labelled No. 1 in Figure 2.

Sample No. 2 was collected September 30, 1968 from the Oyster River. The collection was made near the bridge on Route 4, west of Durham just below the Gaging Station.

TABLE VIII

GC Operating Conditions for Acid Analysis

COLUMN	Length	Dia. 0.02 in. i.d.
	Coating	Conc. wall coated open tubular column
	Support	Mesh
TEMPERATURE	Column, Init. 100°C	Final 190°C
	Rate	Det. 225°C Inj 225°C
CARRIER GAS	Helium	Rate 6°ml/min
	Pressure, Inlet 20 psig	Outlet sub-ambient
	Hydrogen 135 psig	Air 40 psig
DETECTOR	Flame Ionization	
	Sens X1, X2	Recorder range X1, X2
SAMPLE		Size 1 - 3 ul
	Solvent chloroform	Conc. variable

Figure No. 2

Water Sampling Sites

Labelled No. 2 and No. 3 in Figure 2.

Sample No. 3 was collected November 7, 1968 from the Oyster River. The collection site was the same as Sample No. 2. Labelled No. 2 and No. 3 in Figure 2.

Sample No. 4 was collected April 29, 1970 from the Cocheco River. The collection was made west of the bridge on Watson Road. It is labelled No. 4 in Figure 2.

Sample No. 5 was collected November 5, 1970 from the Bellamy River. The collection was made near the bridge on Knox Marsh Road on the southeast side of the road. It is labelled No. 5 in Figure 2.

In all cases approximately ten liters of water were collected. The water was transported to the laboratory for analysis immediately after collection.

MATERIALS

Solvents

n-Pentane: Pesticide grade n-pentane was purchased from Fisher Scientific Company. The solvent was checked for impurities by GC. Three hundred milliliters were evaporated to 2 mls by means of a stream of dried-filtered nitrogen. The remaining 2 mls were transferred to a 3 ml tapered centrifuge tube by means of a transfer pipette. The pentane was evaporated to dryness and the residue taken up in 100 microliters of benzene. The benzene solution was examined by GC using the condition in Table III. No peaks were observed under the specified GC conditions.

Ethanol: Two liters of ethanol were distilled using a 30 cm. Vigreux column. The first 200 mls and last 200 mls were discarded. The center fraction was kept for future use.

Sulfuric Acid: The sulfuric acid was reagent ACS grade purchased from Fisher Scientific Company and used without further purification.

Water: Distilled water from a Barnstead Still was used. The still was protected from air contaminants by a Ventgral filter. De-ionized water was supplied to the still for distillation.

Chemicals: Pyrene and anthracene were purchased from the J. T. Baker Chemical Company. Fluoranthene, benz(a)pyrene and 1,2-benzanthracene were purchased from the Eastman Organic Chemical Company. 2,3-Benzofluorene was obtained from the Aldrich Chemical Company. All chemicals, except fluoranthene, were reagent grade and used as supplied, no additional

purification steps were performed. Fluoranthene was purchased as "practical" grade material.

n-Paraffins: The mixture of reagent grade n-alkanes was purchased from Applied Science Laboratories, Inc. The mixture consisted of the n-$C_{10}H_{22}$, n-$C_{12}H_{26}$, n-$C_{14}H_{30}$, n-$C_{16}H_{34}$ and n-$C_{18}H_{38}$ hydrocarbons.

RESULTS

Introduction

The total ion monitor was used to provide a continuous recording of the separated components entering the ionization chamber. It provided an accurate indication of the best time to obtain a spectrum of the emerging peak. The monitor's output, connected to a recorder, provided a permanent record of the complete chromatogram.

Figure 3 shows the mass spectra for n-hexadecane ($n\text{-}C_{16}H_{34}$) taken at various times as the peak eluted into the mass spectrometer. Figure 3A is the fragmentation pattern of the compound as the peak is first detected in the spectrometer. A good spectrum was obtained except that the parent peak has a very low intensity. This was also the case for the spectrum at the tail end of the peak as seen in Figure 3E. This spectrum also showed additional peaks at the high m/e end which have low intensity or were absent. The best spectra were obtained from the top half of the GC peak. These spectra are essentially identical as seen in Figures 3B, 3C, 3D.

The energy of the electrons used for bombardment was set at 20 eV. Since the ionization potential for helium is 24.8 eV, the ion current for helium was absent from the chromatogram and the peak at m/e 4 was also absent from the mass spectra. The fragmentation of the organic compounds was only slightly different at this lower electron energy. Several comparisons of mass spectra obtained at 70 eV and 20 eV are given in Figures 4, 5, 6 and 7. In all cases studied, the general cracking patterns were the same. A difference in the intensity of the ions which result from processes requiring higher

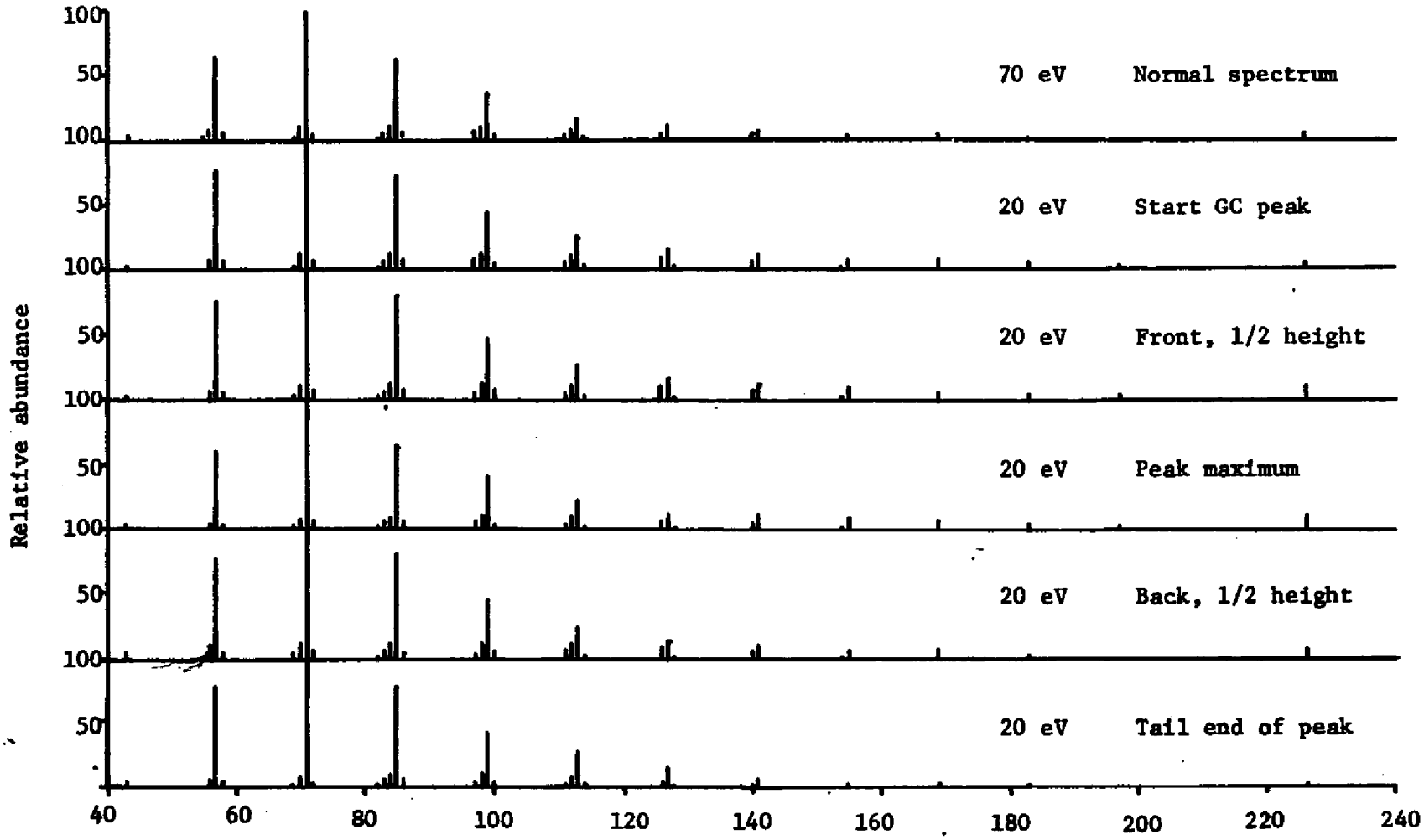


Figure No. 3

Comparison of n-Hexadecane Spectra with Respect to Scanning Position on GC Peak

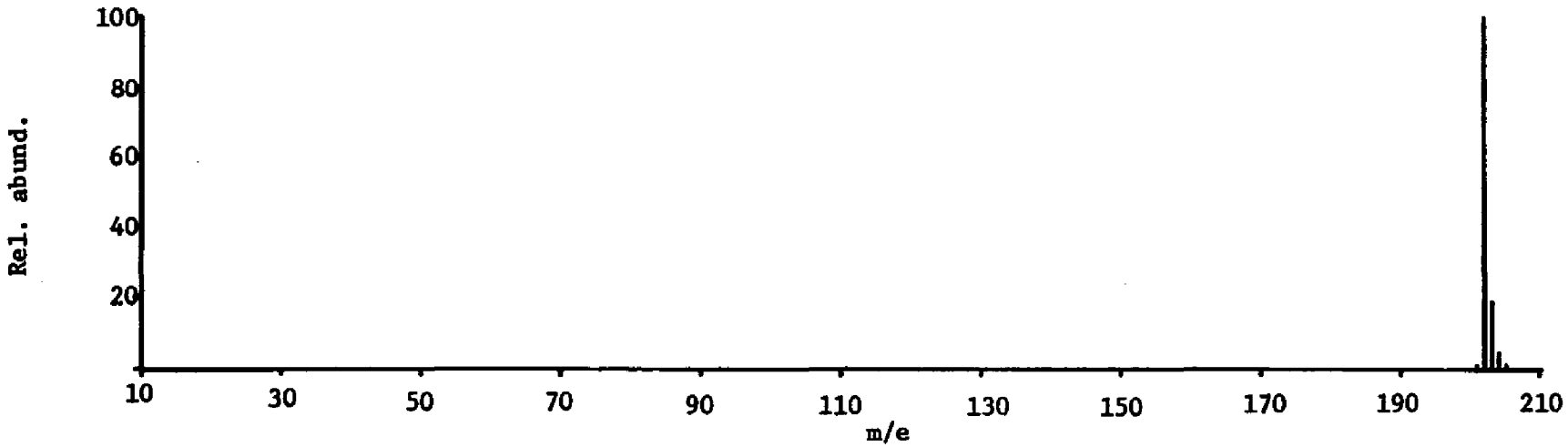


Figure No. 4

Mass Spectrum of Fluoranthene, 20 eV

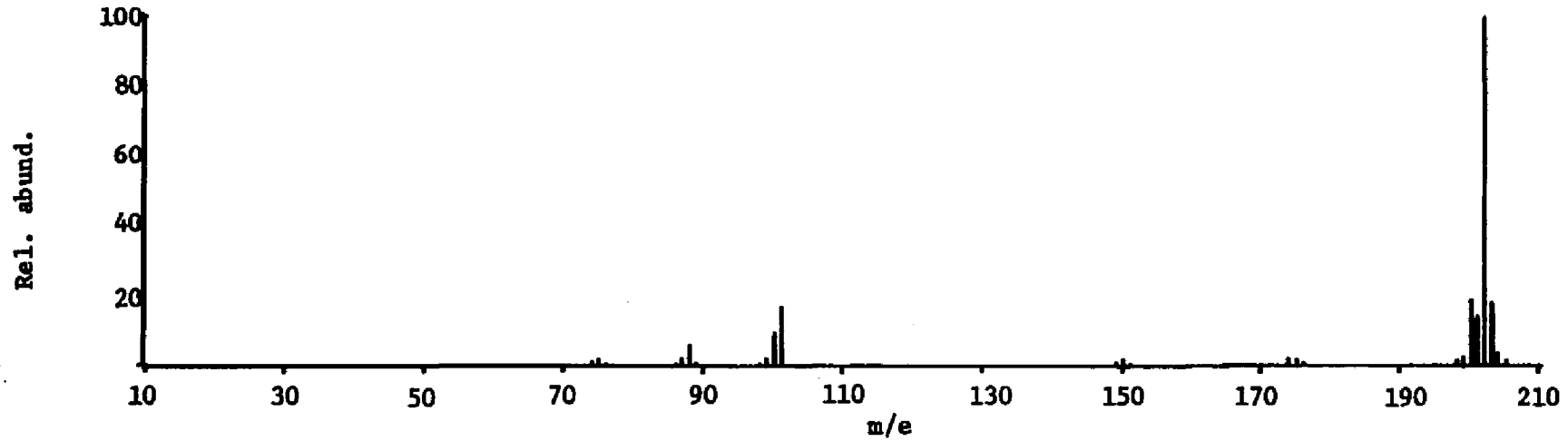


Figure No. 5

Mass Spectrum of Fluoranthene, 70 eV

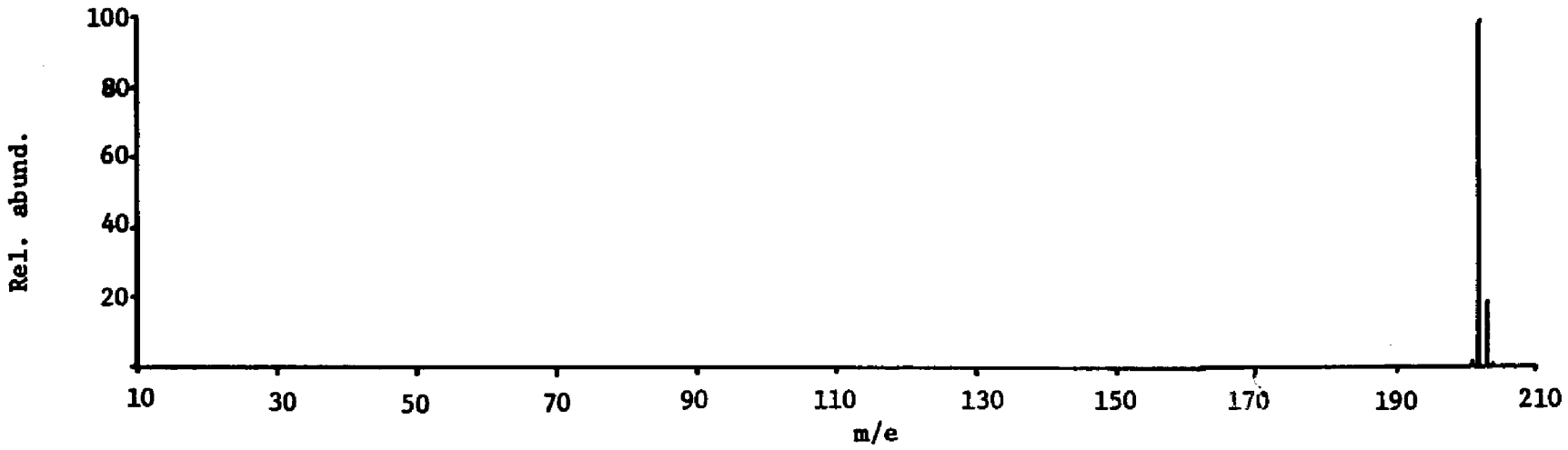


Figure No. 6

Mass Spectrum of Pyrene, 20 eV

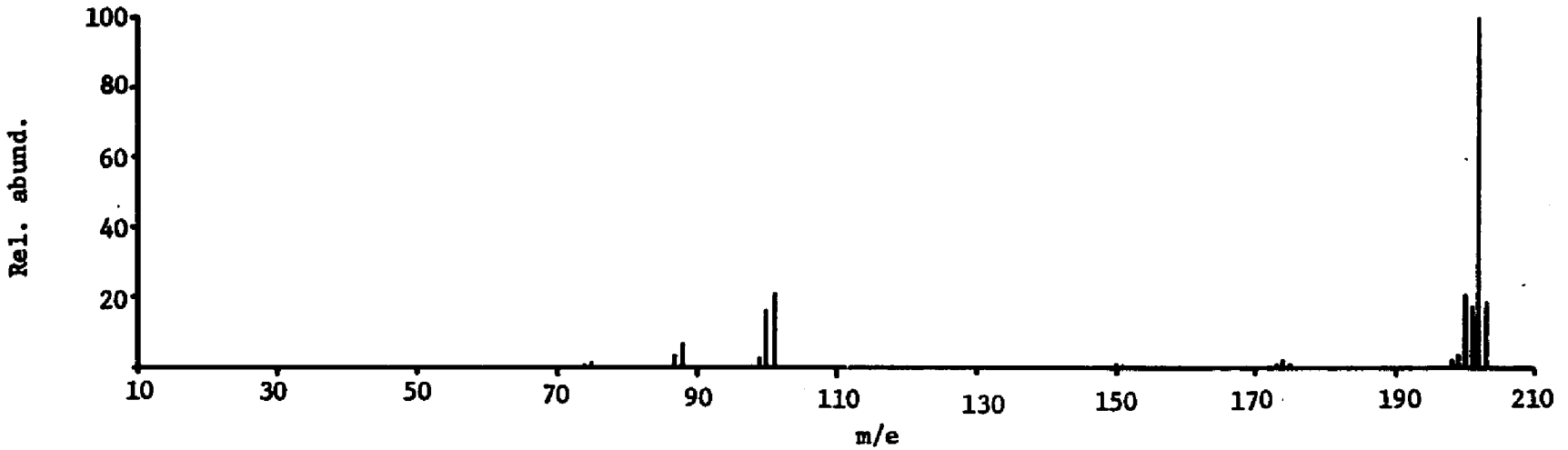


Figure No. 7

Mass Spectrum of Pyrene, 70 eV

energies was observed. This decrease in intensity of the ions which results from high energy fragmentation processes is common at low ionizing (20 eV) voltages.

The 70 eV spectra for pyrene and fluoranthene have a peak at m/e 101. This peak results from the loss of a second electron from the parent ion (m/e 202) resulting in a doubly charged species. The m/e 101 peak was not present in the 20 eV spectra for these compounds because this process occurs at an ionizing potential greater than 20 eV. Likewise the fragmentation patterns for the methyl esters of acids were dominated by the series of ions containing the oxygen atoms. This series is a low energy process and thus favored at 20 eV. The peak at m/e 74 is a rearrangement peak, likely resulting from a low energy process. The m/e 74 peak results from the abstraction of a hydrogen atom from the β-carbon and the breakage of the C-C bond between the β and γ carbon atoms. The m/e 74 peak is the base peak of methyl esters for straight-chain acids greater than C_6. Peaks at m/e 87, 101, etc., are oxygen containing ions at intervals of 14 mass units. The peak at m/e $(M^+ - 31)$ gives an excellent diagnostic peak for methy esters. The peak at m/e $(M^+ - 59)$ is prominent for short-chain esters and decreases rapidly with increasing chain length. When the m/e $(M^+ - 59)$ peak is present it gives an excellent clue to the nature of the alkyl group.

At 20 eV, the low m/e portion of the fragmentation pattern was somewhat reduced in intensity while the high m/e portion was slightly intensified. This was helpful, for it is the high m/e portion which is most useful for identification purposes. This intensity difference can be seen in the spectra for n-dodecane, Figure 8. The peak at m/e 29 is very small in the 20 eV spectra and the m/e 43 is only about 50% that obtained at 70 eV. The parent peak at m/e 170 is slightly increased in intensity at 20 eV, compare with 70 eV.

The reproducibility of spectra will be less at 20 eV than for 70 eV ionization. This was expected, due to the lower stability of the low voltage electrons. Also, the slope of the ionization curve is steep in the 20 eV region and a small change in the electron energy can cause a relatively large change in the ion current intensity. Even at 70 eV, where the ionization potential curve is flat, different instruments and different instrumental conditions can result in drastic spectral changes in certain cases (113). It becomes even more important, at 20 eV, that instrumental conditions be carefully recorded and reproduced.

Figure 8 compares the mass spectra of n-dodecane taken at various scan rates at 20 eV to the spectrum taken at 70 eV taken under optimum conditions. As seen from the figure, a scan rate of 3 seconds, compared to a mone-minute scan, shifted the base peak from m/e 57 to m/e 71. This resulted from two factors. As shown earlier, spectra taken at 20 eV have lower intensities at low m/e ratios. The intensities of the low m/e ratios were decreased with increasing scan rates. This intensity reduction at low m/e ratios was also observed for 70 eV spectra rapidly scanned. The shift in base peak also accounted, in part, for the increased intensity of the parent ion peak when presented in bar graph form. This graphical technique gives the intensities of the various ions as a percentage of the intensity of the strongest peak. The peaks in the high m/e portion were essentially doubled. This resulted because normalization was made on a base peak obtained under 3-seconds scan conditions. This same peak under one-minute scan conditions would be only 50%. This presented no problem, since only the ratio of the intensity was changed and not the fragmentation pattern.

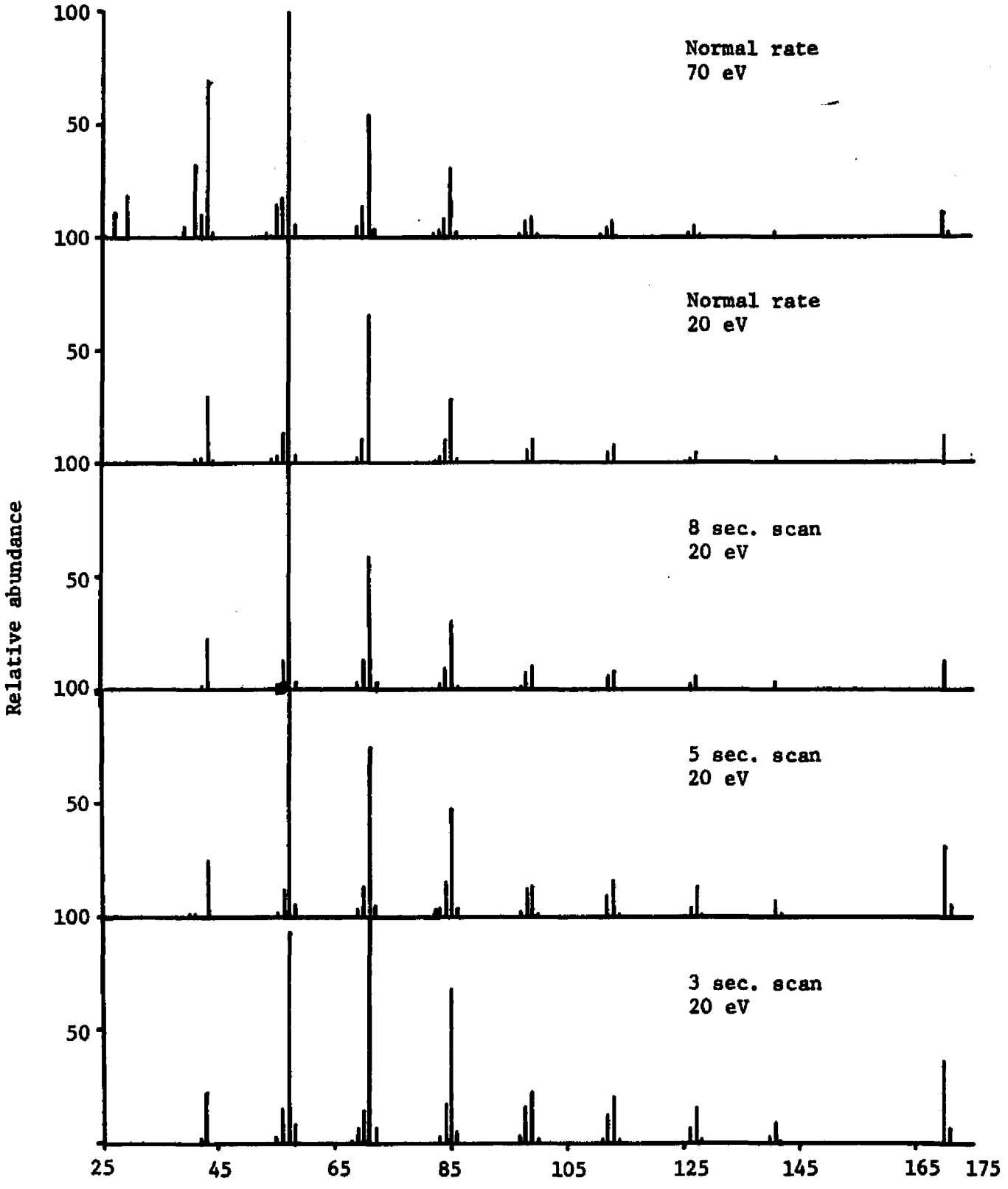


Figure No. 8

Comparison of Mass Spectra with Scanning rate for n-Dodecane

Methylation Method

Several types of acids were examined for ease of methylation using a 10% BCl_3/MeOH reagent. The acids examined were p-hydroxy benzoic acid, benzoic acid, 3-hydroxy-2-naphthoic acid, 1-napththol, 2,4,6-trimethyl benzoic acid, lactic acid, adepic acid, sebacic acid, caproic acid, caprylic acid, palmitic acid, stearic acid, o- and m-toluic acid. The dibasic and monobasic acids were easily methylated using the conditions specified in the experimental section. The extent of methylation was not determined since the intent of developing the procedure was not primarily concerned with specific quantitative determination. For the compounds examined, the phenolic group was not methylated. The carboxyl groups of 2,4,6-trimethyl benzoic acid, o-toluic acid and 3-hydroxy napthoic acid were not methylated with this reagent under the conditions used. This was predicted from the Victor Myer esterification law (114) for sterically hindered positions. Methylation was checked by infrared and mass spectral data after precipitation from a suitable solvent system.

Comparison of Unknown Spectra

Sample spectra were compared against reference spectra obtained from the API Catalog (115), Compilation of Mass Spectral Data (116), which uses the ten most intense peaks in a spectrum, literature sources and spectra obtained in our laboratory, both at 20 eV and 70 eV. When possible more than one source was used because of the variation in spectra under various instrumental conditions. Good agreement between literature spectra and the 20 eV spectra obtained from the analysis existed in some cases. Most literature spectra were obtained at 70 eV, and it should be remembered in comparing these with 20 eV spectra that the lower ionization energy

favors lower energy transitions.

Reference spectra in our laboratory were obtained using the GC as inlet, the liquid-solid inlet and the direct inlet. When possible the GC inlet was used. This technique was preferred because it reproduced essentially identical conditions of background and scanning. It also provided retention times, which could be used as an additional qualitative aid.

Plot of Carbon Number versus Retention Time

When taken at 20 eV, the mass spectra for PNA have relatively simple fragmentation patterns, for this reason it was necessary to have additional qualitative information. The mass spectral patterns for methylanthracene and dimethylathracene consist of m/e (M^+) and m/e (M^+ - 15) for both compounds. Alone, these two peaks could not be used as positive identification. The R_t value for anthracene was available. By plotting R_t versus carbon number (the number of carbon atoms in the molecule) for anthracene, mono- and dimethyl anthracene, the points should fall on a single straight line. Figure 9 shows several such plots. The three points enclosed by circles give the R_t vs 14C, 15C and 16C which represents anthracene $C_{14}H_{10}$, methylanthracene $C_{15}H_{12}$, and dimethyl anthracene $C_{16}H_{14}$. A straight line is obtained through the center of each point. These two methods, GC-MS and R_t vs C plot, give strong evidence for the identification of these three compounds.

Additional plots are also presented, each plot strongly indicates that the individual point is a member of a homologous series. The points enclosed by a square represent a n-alkane series. The points enclosed by a triangle represent several ethyl ester series. The points enclosed by a diamond represents a series of methyl esters of n-acids.

Several plots for the same class of compounds were made when enough points were available. Although the slope from

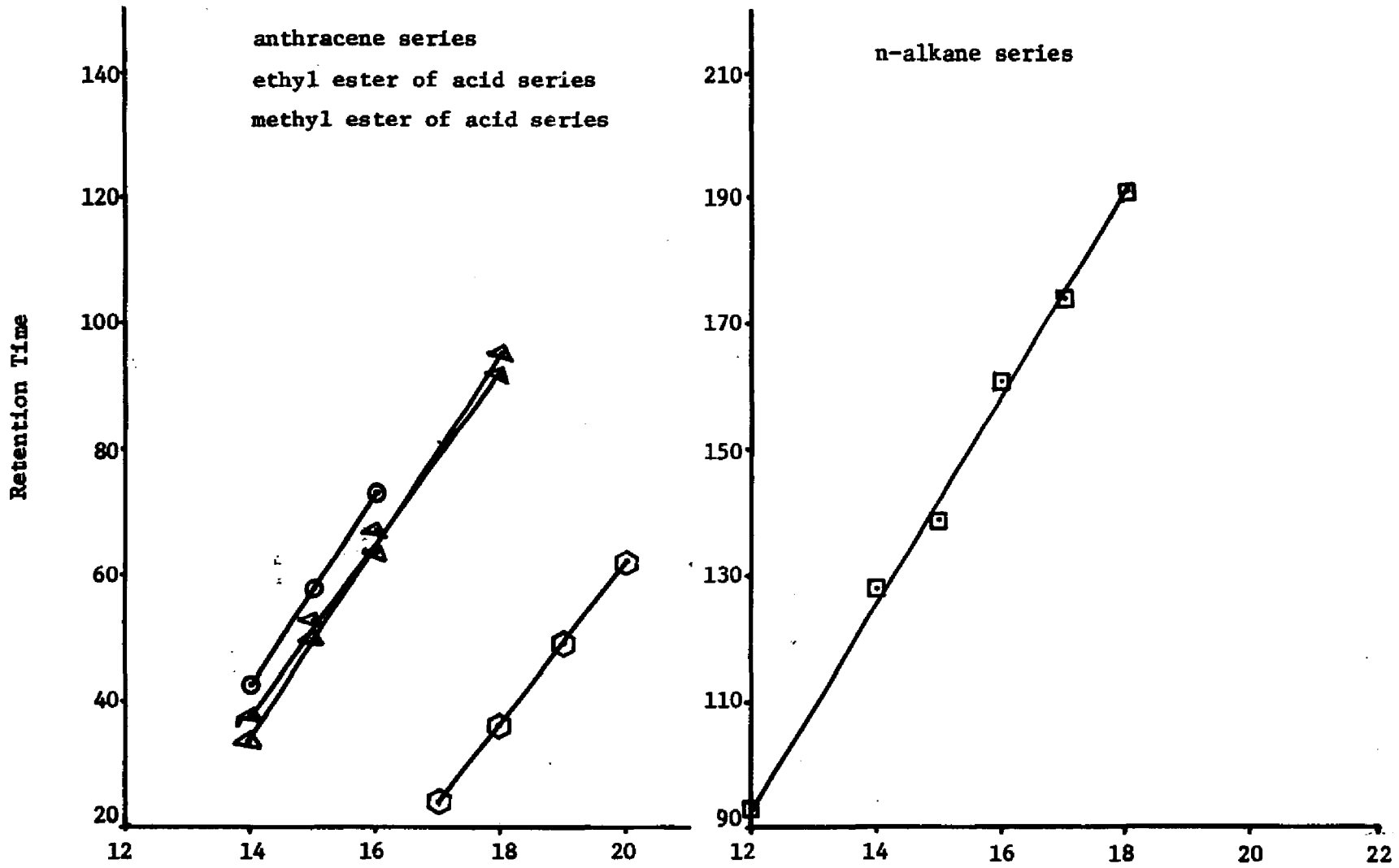


Figure No. 9

Plot Retention Time versus Carbon Number

chromatogram to chromatogram changes, slightly, the lines are in the same general area on the graph for a given type of compound. This provides additional supporting evidence that a given type of compound was being analyzed.

Analysis of PNA

Table IX lists the compounds identified from river water collected April 21, 1968 from the Cocheco River. A number of n-alkanes and three ethyl esters of long chained fatty acids were identified. Fluoranthene was identified, along with three additional cyclic aromatic hydrocarbons. Based upon their mass spectra and the R_t vs C plot, these PNA were tentatively identified as anthracene, methyl anthracene and dimethyl anthracene.

Table X lists the compounds identified from river water collected September 30, 1968 from the Oyster River. All major peaks were identified except one. Its bar graph is presented in Figure 53. The coextractants consist primarily of n-alkanes. The n-$C_{15}H_{32}$ and n-$C_{19}H_{40}$ through n-$C_{25}H_{52}$ hydrocarbons were identified. All of the n-alkanes had adequate molecular ion intensities which greatly helped in their identification.

One PNA was identified as fluoranthene. Its mass spectrum (Figure 4) and retention time agreed with that obtained for a reference compound.

The methyl ester of linlioic acid (Figure 31) was also identified. A good spectrum was obtained and it compared very well with the literature spectrum (117).

An acid having a molecular ion at 284 was also present. The spectrum is presented in Figure 36. Its base peak is at m/e 88; this could represent the ethyl ester or the α-methyl, methyl ester of this acid. Since the m/e (M^+ - 31) was absent and a m/e (M^+ - 43) was present, the compound was tentatively

TABLE IX

Cocheco River, Sample No. 1 April 21, 1968

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Ethyl n-tetradecanoate	256	38	34	---
Anthracene	178	43	21	---
Ethyl n-pentadecanoate	270	53	35	---
Methyl anthracene	192	58	23	---
Ethyl n-hexadecanoate	284	67	36	---
Dimethyl anthracene	206	73	22	---
Fluoranthene	202	78	5	AIP 598
Ethyl n-octadecanoate	312	92	38	---
n-Tricosane	324	118	17	AIP 1859, 1312
n-Tetracosane	338	144	18	AIP 575, 30M
n-Pentacosane	352	185	19	AIP 1313
Solvent impurity	---	206	--	---
n-Hexacosane	366	244	20	AIP 1257

TABLE X

Oyster River, Sample No. 2 September 30, 1968

Compound	Mol. Wt.	Figure No.	Ref. No.
n-Pentadecane	212	10	---
Unidentified	228	53	---
n-Nonadecane	268	13	---
n-Eicosane	282	14	AIP 705, 29M
Ethyl n-hexadecanoate	284	36	---
Fluoranthene	202	5	AIP 958
n-Heneicosane	296	15	---
n-Docosane	310	16	---
Methyl-Linoleate	294	31	117
n-Tricosane	338	17	AIP 1859, 1312
n-Tetracosane	348	18	AIP 575, 30M
n-Pentacosane	352	19	AIP 1313
Solvent impurity	---	--	---

identified as ethyl n-hexadecanoate.

Table XI lists the compounds identified from river water collected November 7, 1968 from the Oyster River. Only one n-alkane was identified, n-$C_{22}H_{46}$. Fluoranthene was also the only PNA identified.

Several ethyl esters of long chained fatty acids were also tentatively identified. This was based upon the presence of the m/e 88 and m/e (M^+ - 43) peaks. The lack of a m/e (M^+ - 31) indicated that the compounds were not methyl esters.

The qualitative plot of R_t vs C gave strong supporting evidence that this set of compounds formed a homologous series since their points fell on a straight line. Figure 9 presents two plots for ethyl esters of acids. The plot with the smaller slope was prepared from data presented in Table IX and the plot with the larger slope was prepared from Table XI.

The other compound to be positively identified was dibutyl phthalate. Its very strong peak at m/e 149 and the weaker peaks at m/e 223 and 104 give strong evidence for the ortho isomer.

Table XII lists the compounds identified from river water collected April 29, 1970 from the Cocheco River. This sample contained a large number of peaks in the chromatogram, many of which did not give adequate mass spectra for identification. This was due, in part, to their low concentration. The bulk of the organic component for this sample was made up of n-alkanes. Methyl anthracene, fluoranthene and dibutyl phthalate were also present.

Summary of the Analyses of the Pentane Extractions

Four samples taken from New Hampshire rivers were qualitatively analyzed by a GC-MS system. Three classes of compounds were identified. The n-alkanes made up the bulk of

TABLE XI

Oyster River, Sample No. 3 November 7, 1968

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Ethyl n-tetradecanoate	256	34	34	---
Unidentified	228	40	53	---
Ethyl n-pentadecanoate	270	50	35	AIP 1006
n-Dibutyl phthalate	278	60	26	
Ethyl n-hexadecanoate	284	64	36	---
Fluoranthene	202	73	5	AIP 958
Ethyl n-octadecanoate	312	95	38	---
n-Docosane	310	97	16	AIP 1311
Solvent Impurity	---	--	--	---

TABLE XII

Cocheco River, Sample No. 4 April 29, 1970

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
n-Heptadecane	240	24	11	AIP 1006
n-Octadecane	254	36	12	AIP 574
Unidentified	228	39	53	---
n-Nonadecane	268	49	13	AIP 592
n-Dibutyl phthalate	278	58	43	
n-Eicosane	282	62	14	AIP 705, 29M
Ethyl n-hexadecanoate	284	64	36	---
Dimethyl anthracene	206	69	22	
Fluoranthene	202	78	5	AIP 598
n-Tricosane	324	133	17	AIP 1312

the components identified. Eleven different n-alkanes were identified ranging from n-pentadecane to n-hexacosane. Not all of these alkanes were identified in each river sample. The samples collected from the Cocheco River, April 1968 and April 1970, contained respectively four and five identified n-alkanes (see Tables IX and X for specific compounds). In the 1968 sample, four high molecular weight n-alkanes ($C_{23}H_{78}$ through $C_{26}H_{54}$) were identified. The 1970 sample contained n-alkanes throughout the molecular weight range eluted under the GC conditions used for the analysis.

The September 1968 Oyster River sample contained eight identified n-alkanes while the November 1968 sample contained only one identified n-alkane. It is impossible to determine from the limited data a seasonal relationship or a clear indication as to differences in contamination level between the two rivers for this class of compounds.

Esters made up the second large group of compounds identified. Four different ethyl esters of n-alkyl acids were identified; at least one of these esters was found in each river sample. Two additional esters were identified; in the 1970 Cocheco River sample, n-dibutyl phthalate was identified and in the 1968 Oyster River sample, methyl linoleate was identified. Again, no seasonal relationship or difference in contamination level between rivers can be made.

The final class of compounds to be identified was the polynuclear aromatic hydrocarbons. Four different PNA were identified. Fluoranthene was the only PNA to be found in all four samples. Anthracene, methyl- and dimethyl anthracene were identified only in the 1968 Cocheoc River sample. No correlation between rivers and seasons can be drawn.

The four PNA identified by the GC-MS were all highly fluorescent compounds and should be detectable by a TLC-fluorescent method. The inertness of n-alkanes and the fact

that pentane, hexane and other alkanes are recommended solvents for solution fluorescent methods, even high concentration of this class of compounds should not constitute an interference in fluorescence analysis. This may not be true for the esters. The presence of unsaturation, the oxygen atoms and their possible interaction with active sites on the TLC adsorbent could cause this class of compound to constitute an interference in the TLC-fluorescent method. Additional studies will be required to clear up this point.

The banks or the tributaries of the Oyster River do not contain towns, cities or industrial complexes above the sampling site. The types of compounds identified from the Oyster River are not associated with agricultural fertilizers or pesticides. It must therefore be concluded that the compounds identified in this study originated from natural sources.

Summary of the Analyses of the Ion Exchange Extraction

Several elutions of the ion exchange column were made. This resulted in fractionation of the types of acids eluted from the resin. The first washing of the resin was made with 1N NaOH. The NaOH elution was further fractionated into the chloroform soluble acids and the n-butanol soluble acids.

These two acid fractions were then examined by a tandem GC-MS system. The chloroform fraction is presented in Table XIII. The mass spectra for compounds listed in Table XIII are presented in the Appendix. The type of acids extracted with chloroform were not generally n-alkyl fatty acids. Only one such acid was identified, methyl n-tetradecanoate. This acid represents the highest molecular weight compound identified in the chloroform fraction. Only one additional acid ester was identified, methyl cinnamate. Seven unidentified spectra are presented. Because the molecular weights do not steadily

TABLE XIII

Bellamy River, Sample No. 5 Chloroform Extract

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Unidentified	98	14	48	---
Unidentified	138	--	49	---
Unidentified	154	48	44	---
Unidentified	138	55	50	---
Unidentified	178	63	46	---
Unidentified	160	109	51	---
Methyl cinnamate	162	115	40	---
Unidentified	178	--	45	---
Unidentified	200	188	41	---
Methyl n-tetradecanoate	242	202	25	---

increase with increasing R_t, it is assumed that more than one type of acid was present. A prominent feature in the fragmentation patterns for the unidentified acid esters was the m/e (M^+ - 15) peak. This indicated that a favored fragmentation was the loss of a methyl group from the molecular ion. Since a m/e (M^+ - 31) peak was not observed, it can be assumed that the methylated carboxyl group was not attached directly to an aromatic system, since the m/e (M^+ - 31) would be expected in the fragmentation pattern for aromatic acid esters.

Table XIV presents the acid resulting from the n-butanol extraction. This fraction consists essentially of straight chain fatty acids. Nine such acid esters were identified, ranging from methyl n-decanoate to methyl n-nonadecanoate. Three unsaturated fatty acid esters were also identified: methyl octadecantrienoate, methyl octadecandienoate and methyl octadecenoate. Two aromatic acid esters were identified: n-dibutyl phthalate and dimethyl terephthalate. The n-butanol fraction differs significantly from the chloroform fraction.

It can be seen from Tables XIII and XIV that several of the lower molecular weight compounds were incompletely extracted by chloroform. It can also be seen that the chloroform solvent, often used for the extraction of acids, did not adequately extract the high molecular weight n-alkyl acids. The bulk of this class of compounds appeared in the n-butanol extract. Yet the conditions of the chloroform extract, acidic pH and high salt concentration, implies good conditions for high extraction efficiency of acids. It is this type of qualitative information which is needed to understand and devise better procedures as required in the quantitative analysis of complex pollution analysis.

N-dibutyl phthalate was the only strange "ester" among the esters identified. Its presence can be explained as

TABLE XIV

Bellamy River, Sample No. 5 n-Butanol Extract

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Unidentified	138	30	49	---
Unidentified	140	39	none given	---
Unidentified	170	49	none given	---
Methyl n-decanoate	186	53	39	117
Unidentified	154	67	none given	---
Unidentified	156	73	none given	---
Dimethyl phthalate	194	86	47	---
Methyl n-dodecanoate	214	93	24	117
Unidentified	212	97	none given	---
Unidentified	226	105	none given	---
Methyl n-tetradecanoate	242	128	25	117
Methyl n-pentadecanoate	256	139	30	117
n-Dibutyl phthalate	278	152	43	117
Methyl n-hexadecanoate	270	161	26	117
Methyl n-heptadecanoate	284	174	27	117

Table XIV cont.

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Methyl octadecantrienoate	292	185	32	117
Methyl octadecandienoate	294	185	31	117
Methyl octadecenoate	296	185	33	117
Methyl n-octadecanoate	298	191	28	117
Methyl n-nonadecanoate	312	---	29	
Unidentified	314	---	52	

follows. The phthalic acid isomers were extracted by n-butanol. The solvent, n-butanol, was removed by evaporation with a small amount of heat required. During the heating process the ortho-phthalic acid was converted to the anhydride, which reacted with the alcohol to give the n-dibutyl ester of phthalic acid. The meta- and para-phthalic acids are not so easily converted to the anhydride. It was for this reason that the n-butyl ester of o-phthalic acid was detected and the methyl esters of the m- and p-isomers of phthalic acid were detected.

Mass spectra were also obtained for three components which were eluted as a single symmetrical peak from the GC. The components were identified as the methyl ester of linolenic acid, which has three double bonds each separated by a methylene group, methyl esters of linolic acid, which has two double bonds separated by a single methylene group and the methyl ester of oleic acid, which has only one double bond. By taking sequential mass spectra as the peak eluted into the spectrometer, the mass spectrum for each component could be obtained. The earliest spectra were characteristic of the methyl ester of linolenic acid. Positive identification for these three acid esters was made using this technique of successive scanning. Several other intense chromatographic peaks were examined by this technique. These peaks appeared to be single component peaks.

The fragmentation patterns for the compounds identified have been discussed in several sources (118,119,120) and will not be discussed here.

SUMMARY

Two methods for the extraction and analysis of organic compounds in fresh water have been developed. In the first method, water was continuously extracted with n-pentane. Since PNA were the compounds of principal interest, the constancy of the fluorescent intensity of the pentane was used to determine the completion of the extraction. The pentane volume was reduced and extracted with a strong acid solution to remove fluorescing basic materials. The pentane was dried and reduced to dryness. The residue was dissolved in benzene and examined by GC-MS.

A second method has been presented for the separation of acidic organic materials into a number of fractions. Humic acids were removed from the aqueous phase by precipitation and filtration from a strongly acidic solution. The acidic aqueous solution was passed through a strong anion exchange resin to further isolate acidic organic compounds. The resin was eluted with a strong basic solution. A second washing of the resin with a strong salt solution resulted in the elution of additional material. The effluent were made acidic and extracted with a nonpolar and a polar solvent. These fractions were methylated and examined by GC-MS.

The combination of a gas chromatograph with a mass spectrometer offers a number of advantages. The extensive purification of a compound to be analyzed is usually not required with the GC-MS system. It is also possible to identify many contaminants by their mass spectra, if required. Since a mass spectrum can be taken every three seconds, the effluent passing through the ion source can be observed almost

continuously. Thus, components need not be completely resolved in the gas chromatograph in order to be identified. The number of samples which can be analyzed by a GC-MS system is thus greatly increased. The vacuum is not broken to introduce the sample; thus there is no waiting for pump down. The GC system introduces very small amounts of sample which can be easily removed by the vacuum pumping system. High background and most memory effects are usually absent from successive spectra without long waiting periods between runs. The low ionizing voltage increases the probability that the molecular ion will be present. Thus an exact molecular weight for most compounds can be obtained.

Certain disadvantages exist with the system. Compounds which are thermally unstable or decompositions which are metal catalyzed cannot normally be introduced by this technique due to the long periods spent at elevated temperatures and large metal surface areas in the system. The compounds to be introduced to the mass spectrometer are also limited by the maximum recommended temperature of the liquid phase in the gas chromatographic column.

The low ionization potential can substantially reduce the number of ions observed in a fragmentation pattern for certain types of compounds. This reduction in the number of observed peaks greatly reduces the chance for the positive identification of a compound from its mass spectrum. The amount of data obtained by the GC-MS system presents another problem. Depending upon the complexity of the chromatogram and the nature of the information sought, one chromatogram could provide as much as a month's work in data reduction by manual methods and the chances for error in the manual reduction of data is very high.

Irrespective of the above mentioned disadvantages, the GC-MS system offers an excellent tool for the analysis of

complex samples. The computerization of this system for data reduction and file searching will greatly enchance the capabilities of the tandem GC-MS approach.

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A P P E N D I X

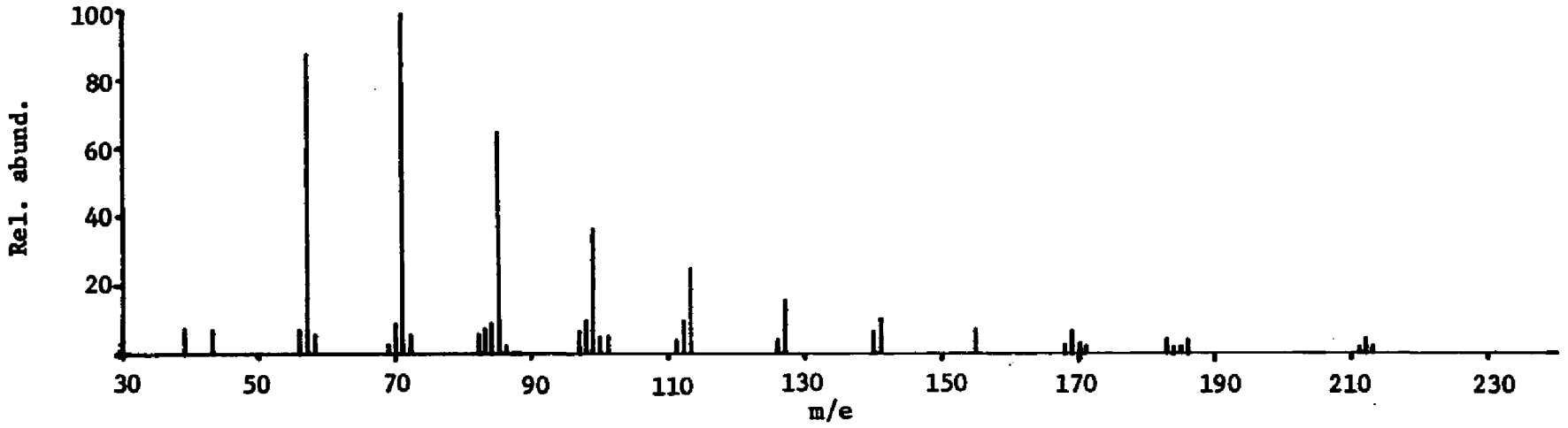

Figure No. 10

Mass Spectrum of n-Pentadecane m/e 212

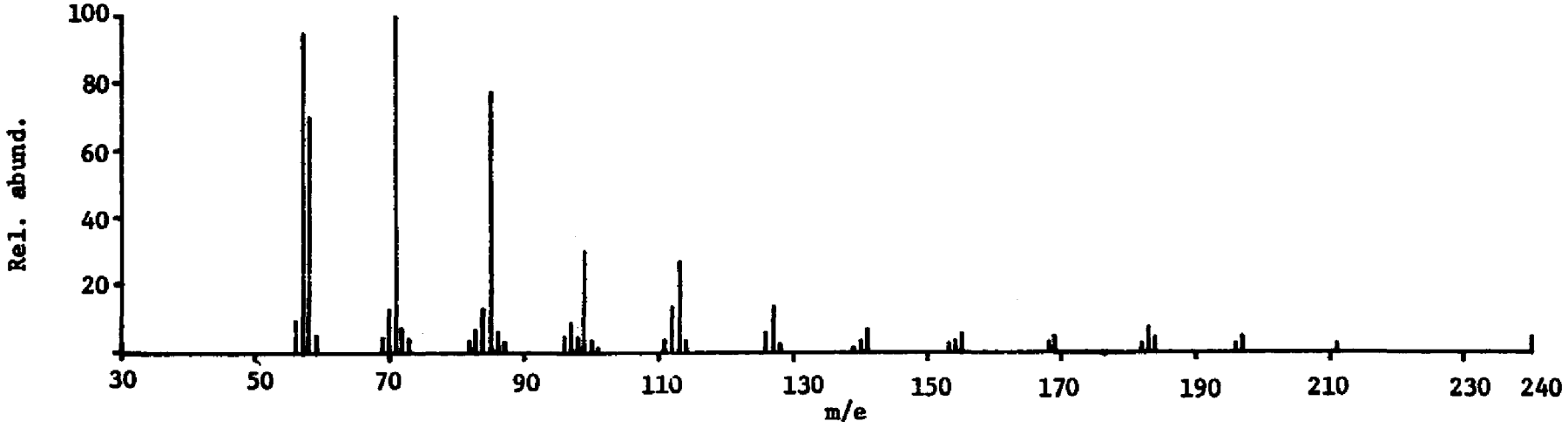

Figure No. 11

Mass Spectrum of n-Heptadecane m/e 240

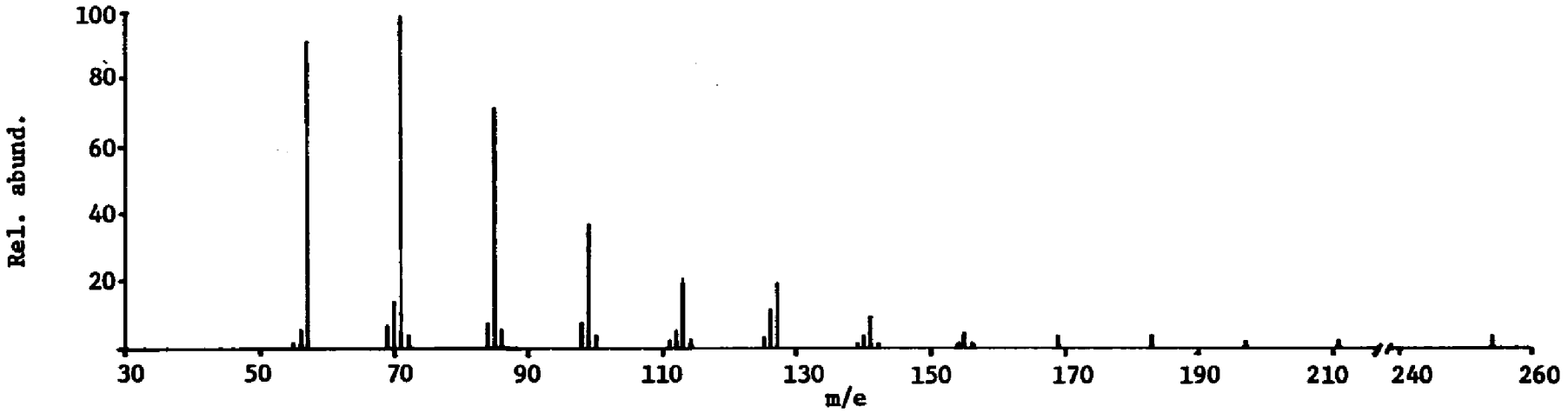

Figure No. 12

Mass Spectrum of n-Octadecane m/e 254

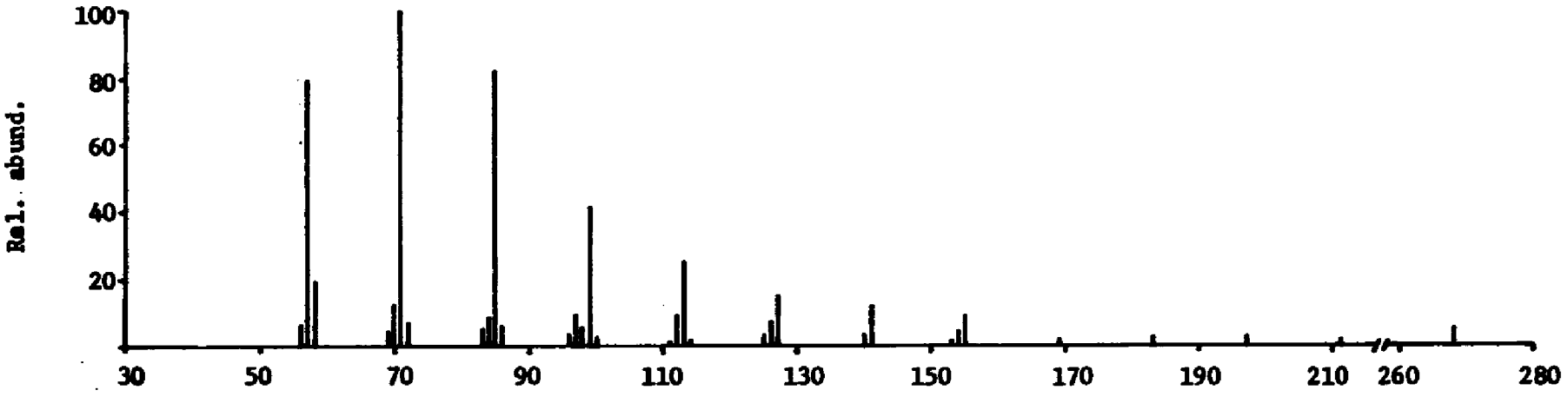

Figure No. 13

Mass Spectrum of n-Nonadecane m/e 268

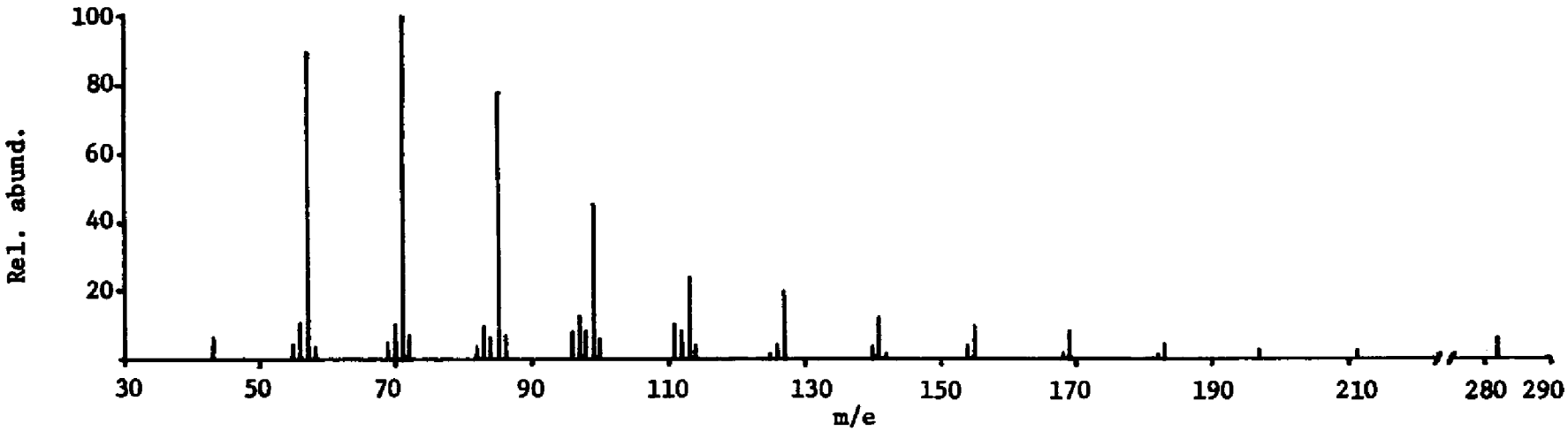

Figure No. 14

Mass Spectrum of n-Eicosane m/e 282

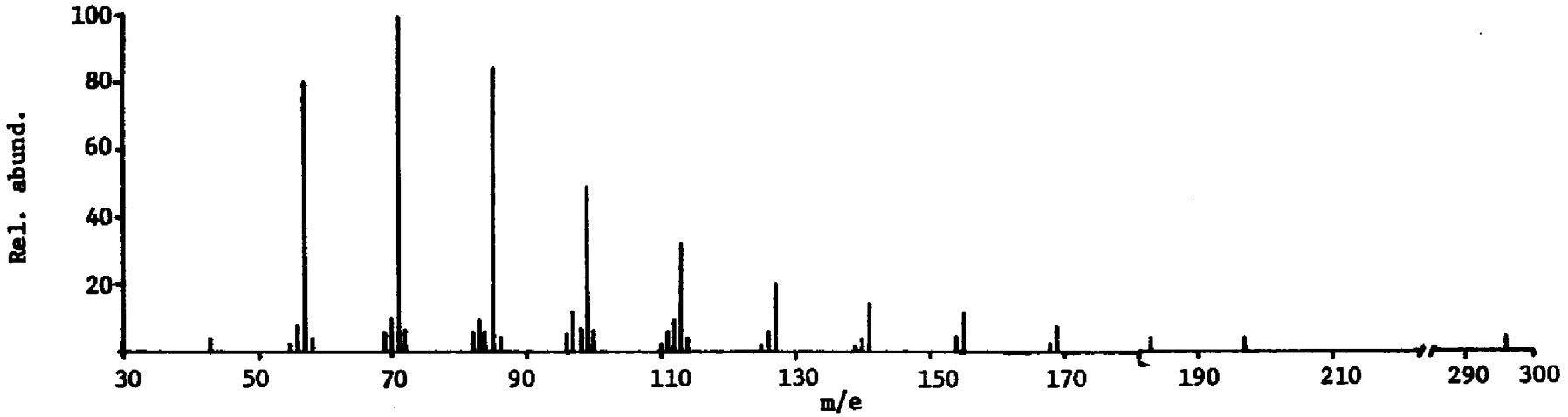

Figure No. 15

Mass Spectrum of n-Heneicosane m/e 296

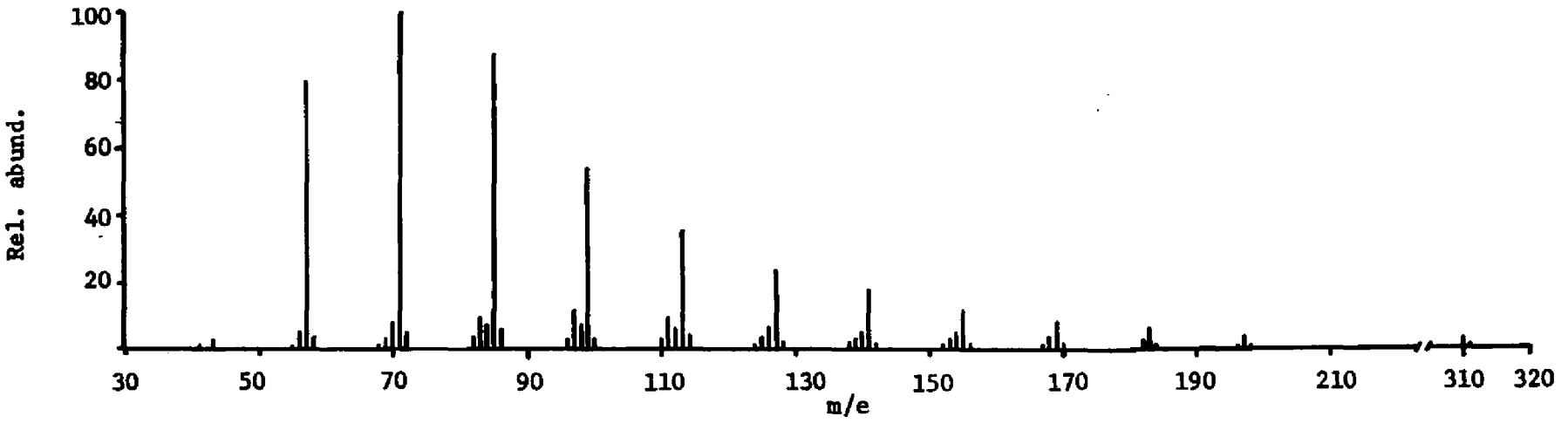

Figure No. 16

Mass Spectrum of n-Docosane m/e 310

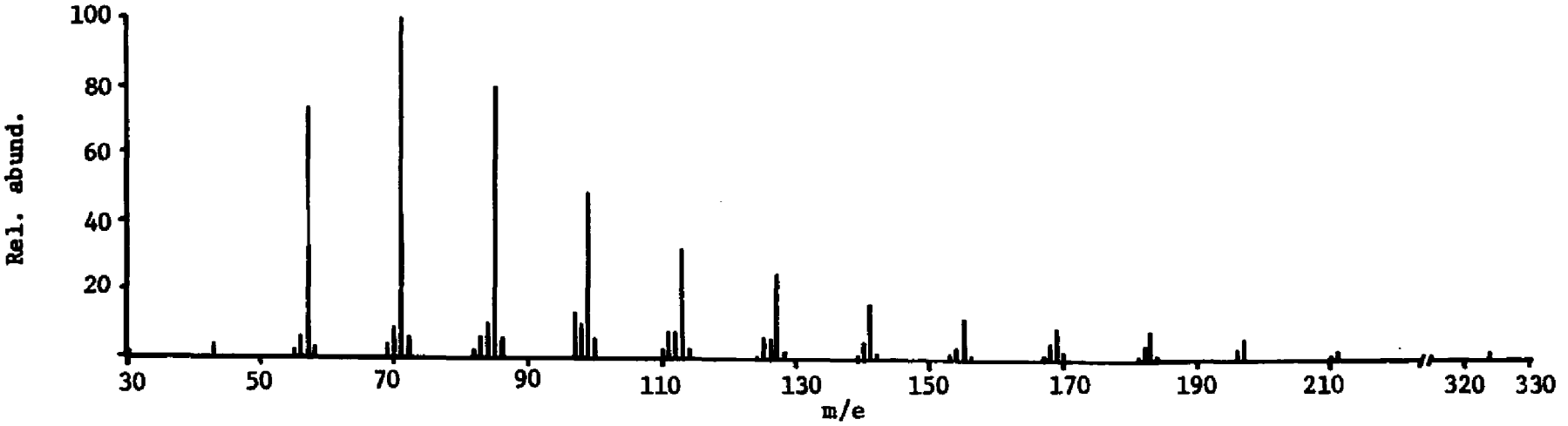

Figure No. 17

Mass Spectrum of n-Tricosane m/e 324

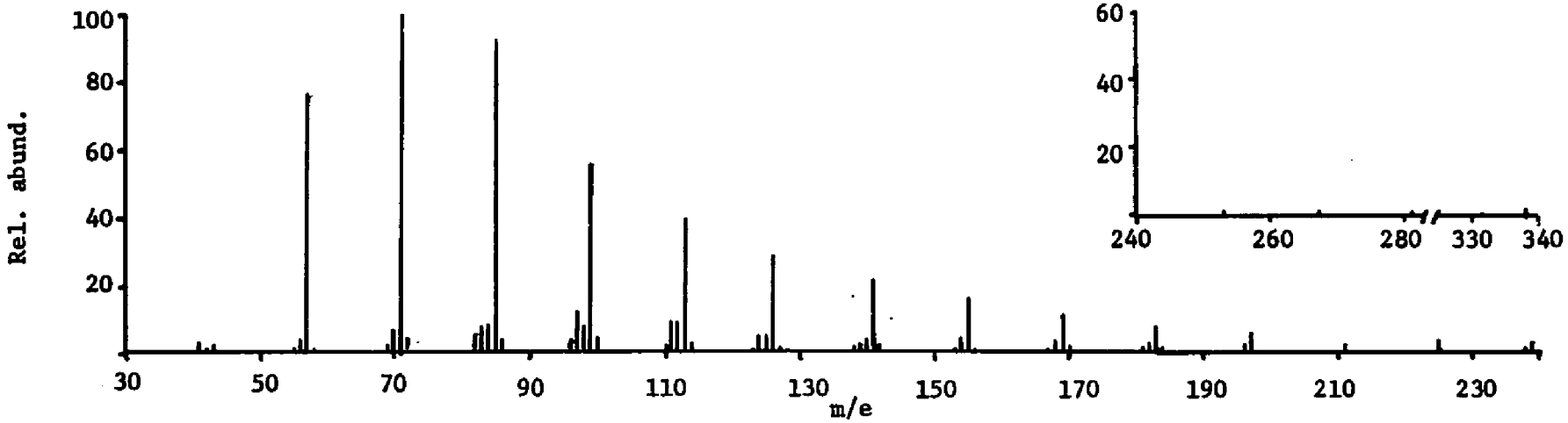

Figure No. 18

Mass Spectrum of n-Tetracosane m/e 338

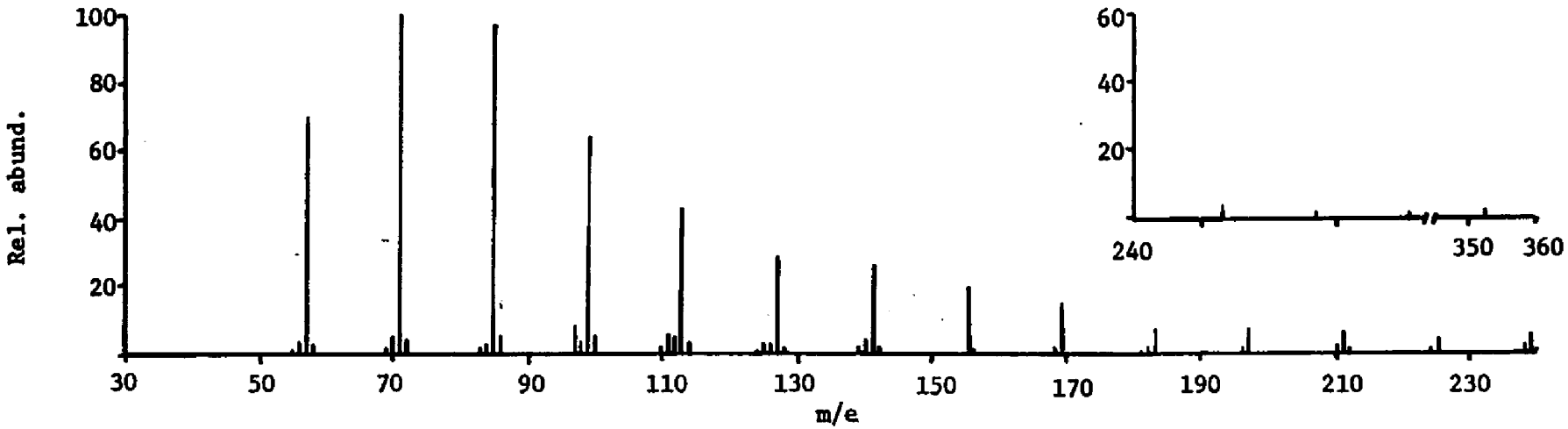

Figure No. 19

Mass Spectrum of n-Pentacosane m/e 352

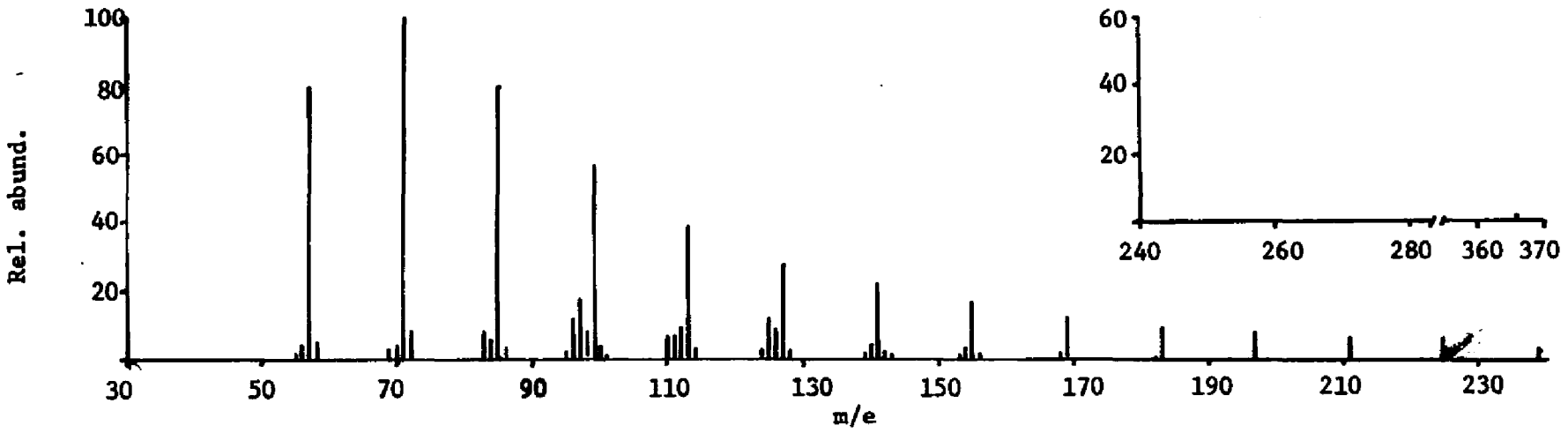

Figure No. 20

Mass Spectrum of n-Hexacosane m/e 366

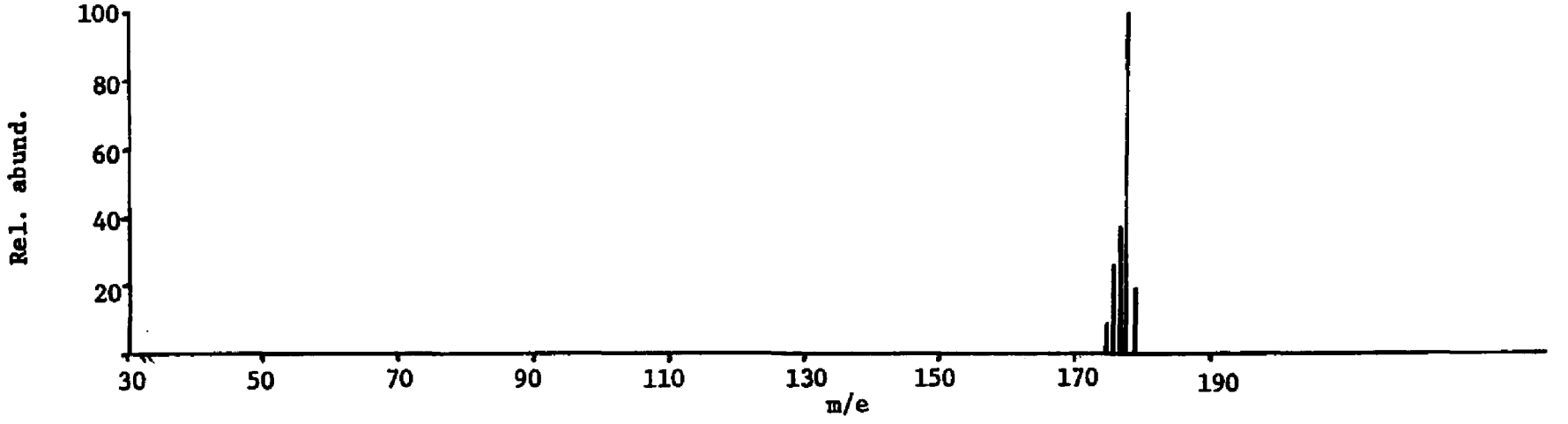

Figure No. 21

Mass Spectrum of Anthracene m/e 178

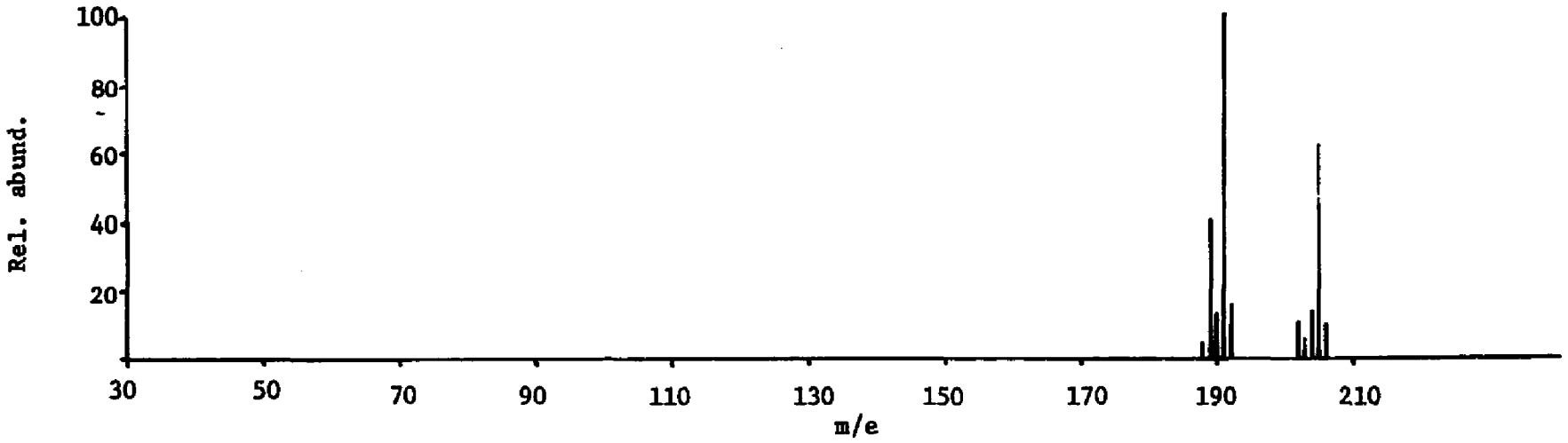

Figure No. 22

Mass Spectrum of Dimethyl Anthracene m/e 206

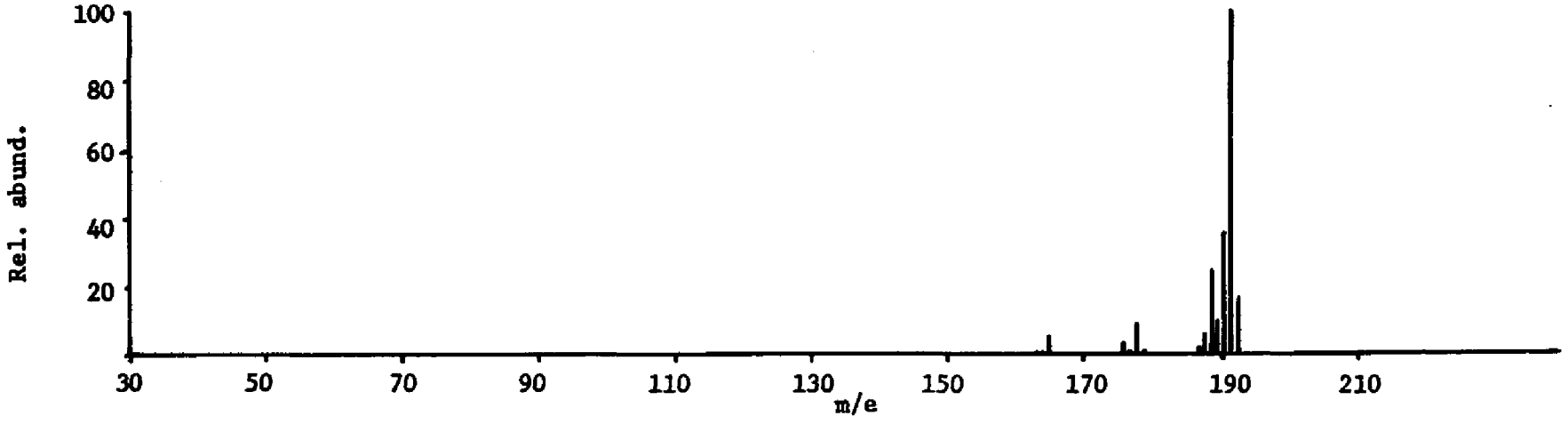

Figure No. 23

Mass Spectrum of Methyl Anthracene m/e 192

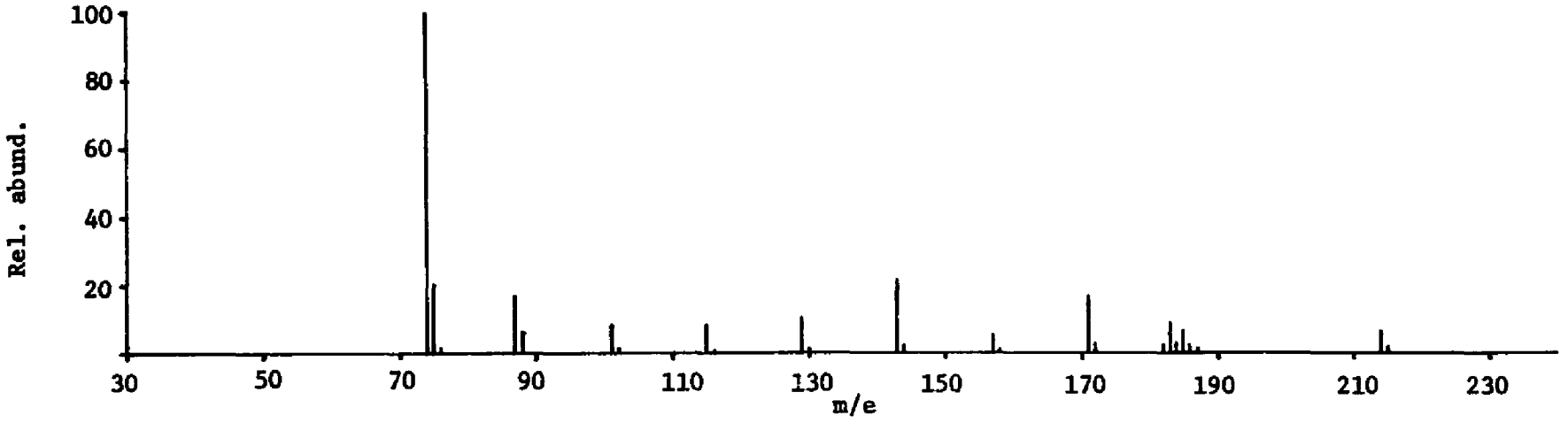

Figure No. 24

Mass Spectrum of Methyl n-Dodecanoate m/e 214

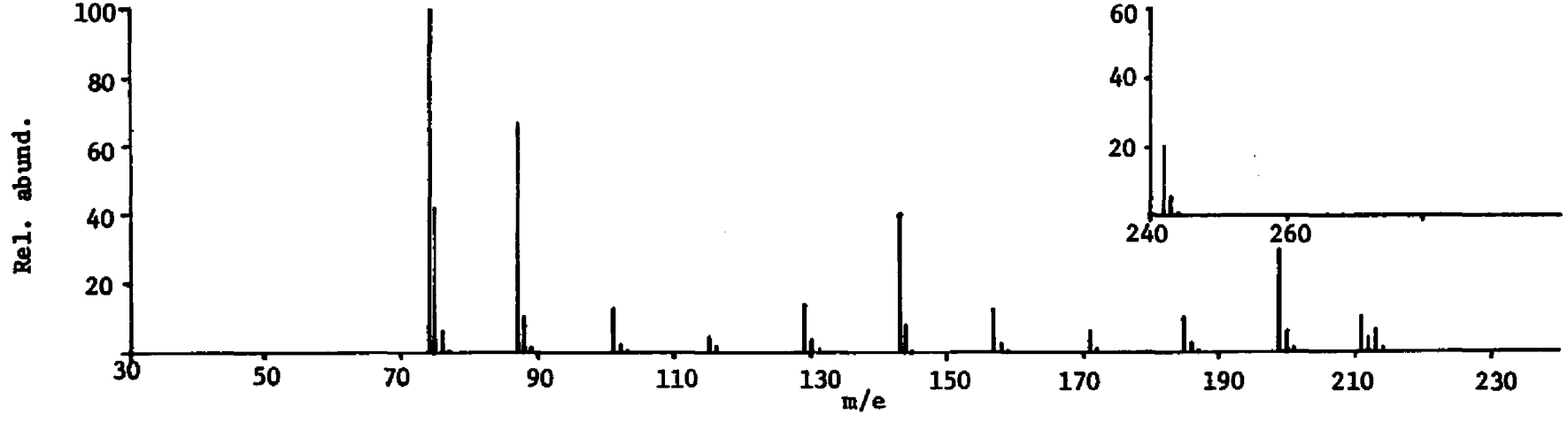

Figure No. 25

Mass Spectrum of Methyl n-Tetradecanoate m/e 242

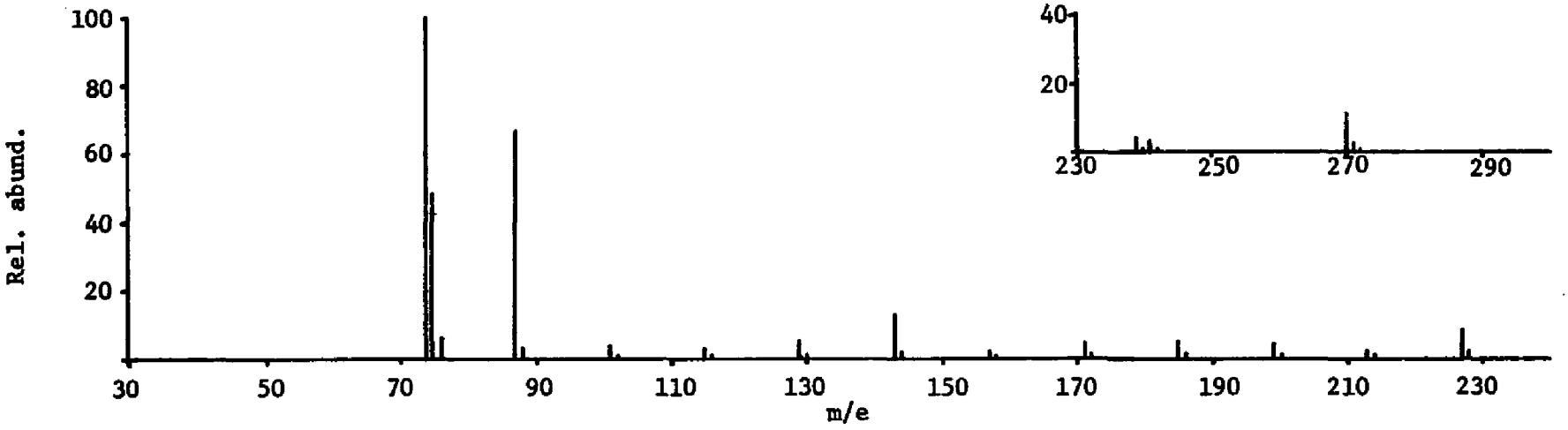

Figure No. 26

Mass Spectrum of Methyl n-Hexadecanoate m/e 270

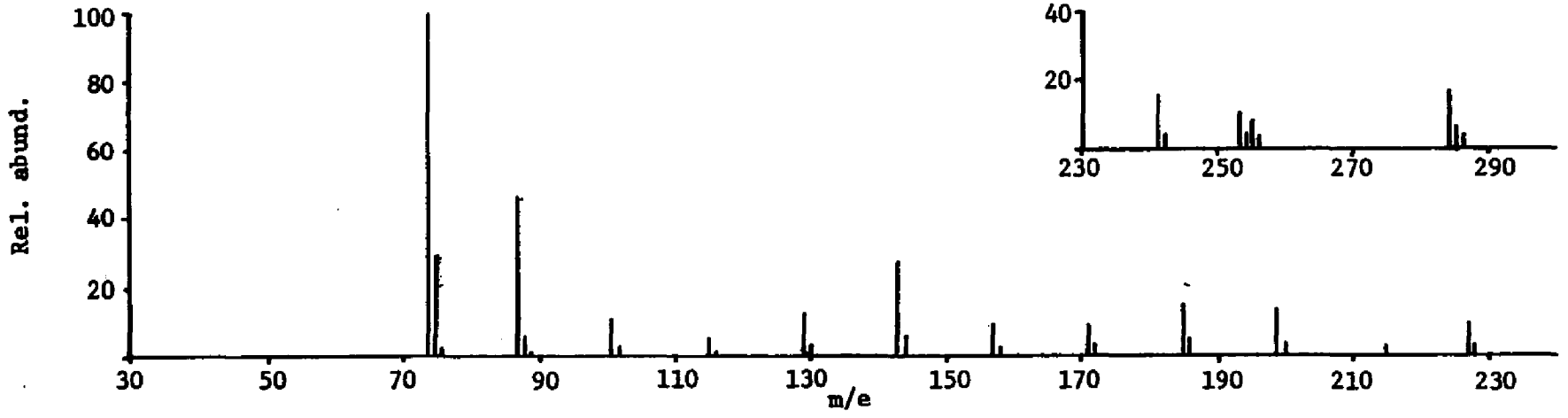

Figure No. 27

Mass Spectrum of Methyl n-Heptadecanoate m/e 284

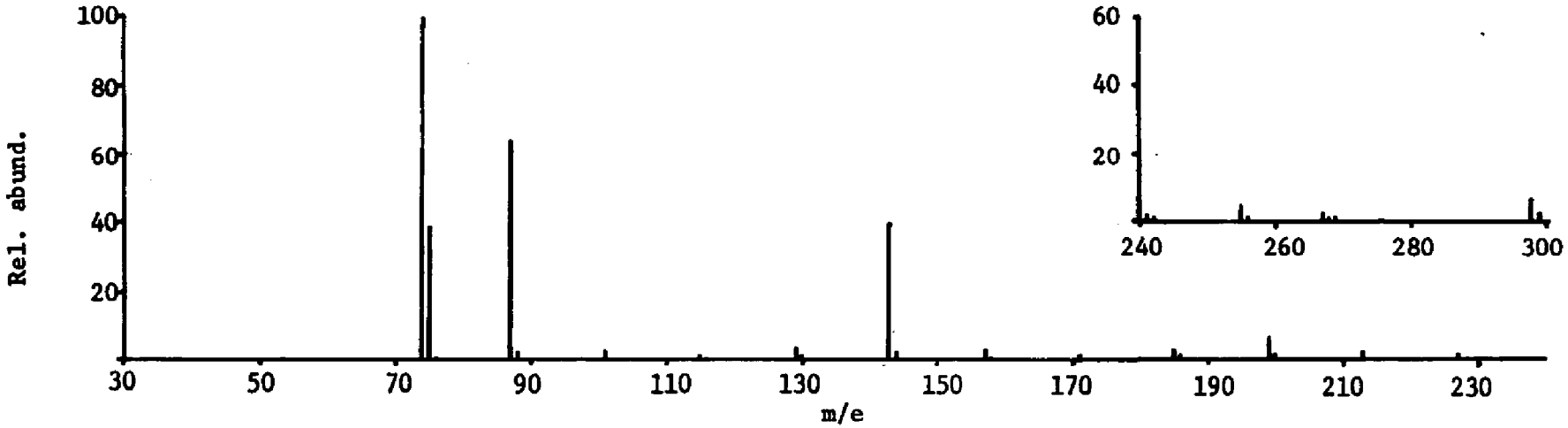

Figure No. 28

Mass Spectrum of Methyl n-Octadecanoate m/e 298

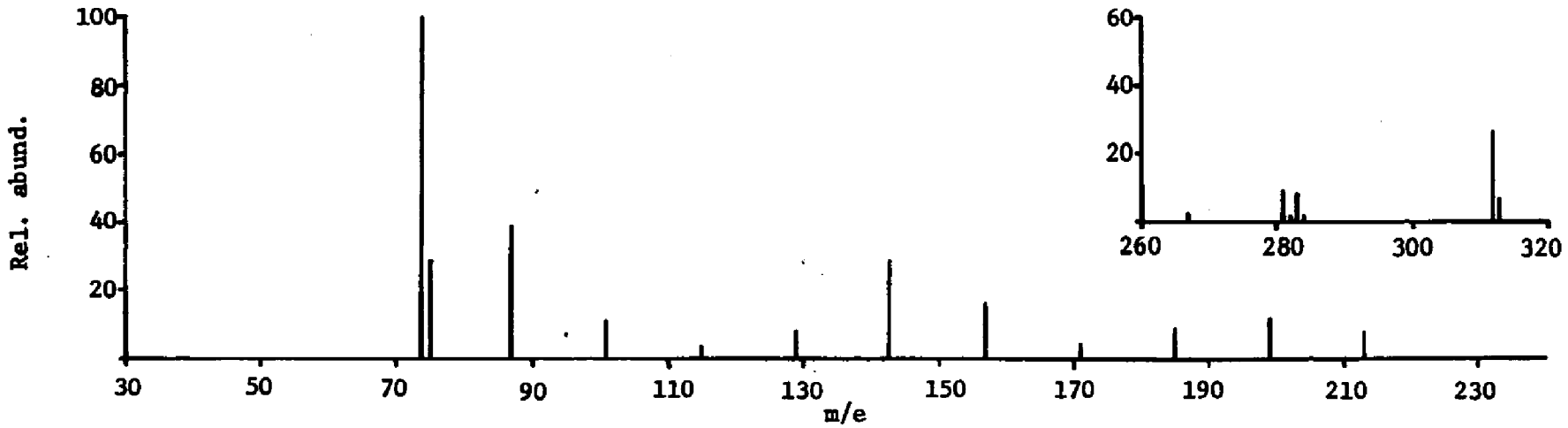

Figure No. 29

Mass Spectrum of Methyl n-Nonadecanoate m/e 312

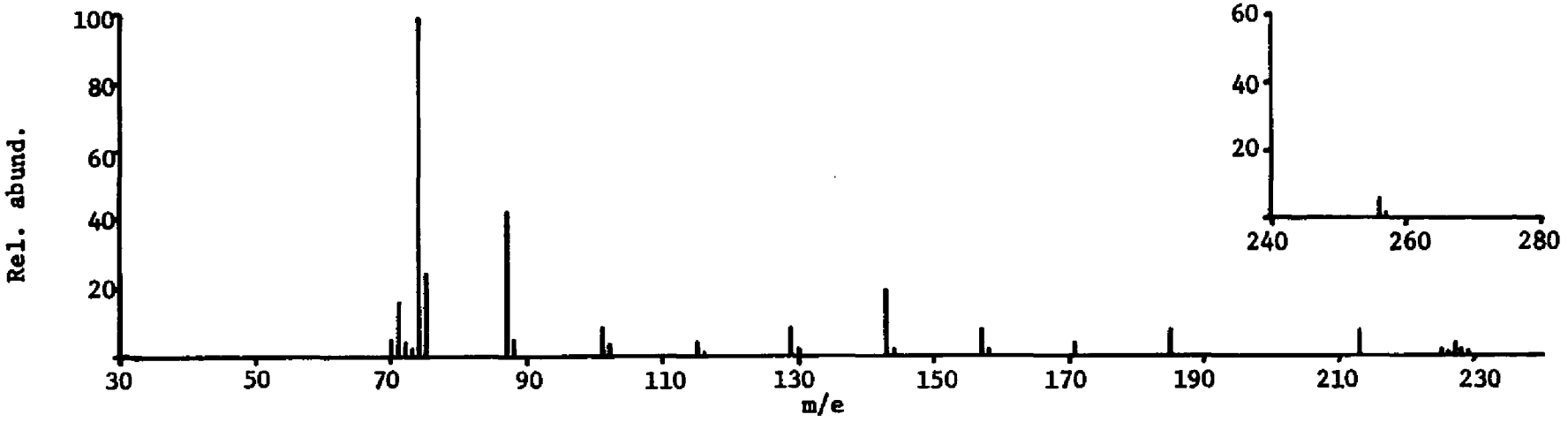

Figure No. 30

Mass Spectrum of Methyl n-Pentadecanoate m/e 256

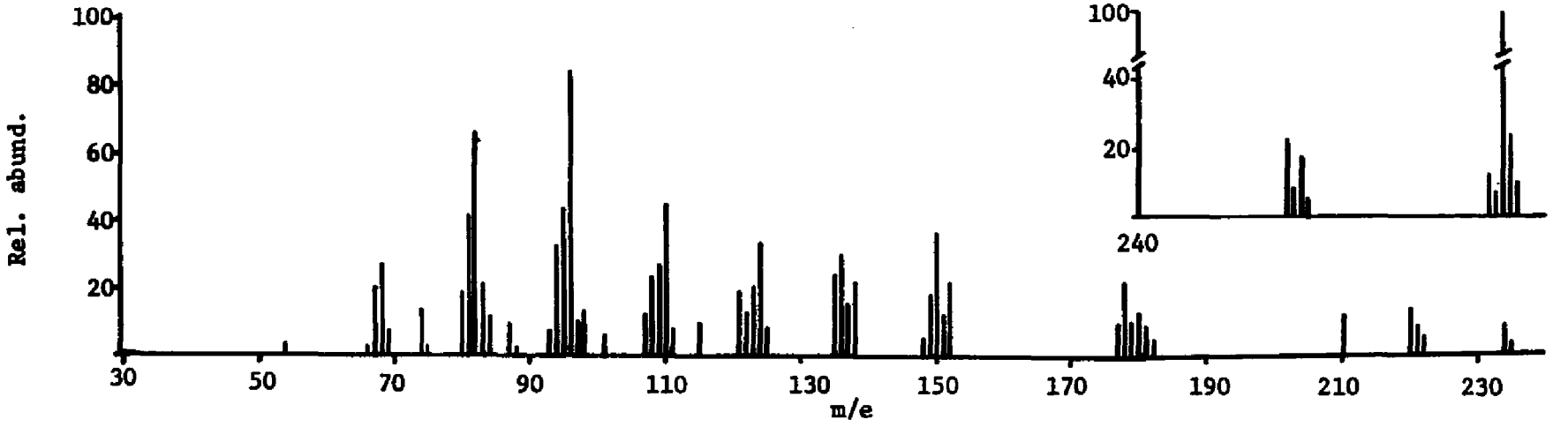

Figure No. 31

Mass Spectrum of Methyl Octadecandienoate m/e 294

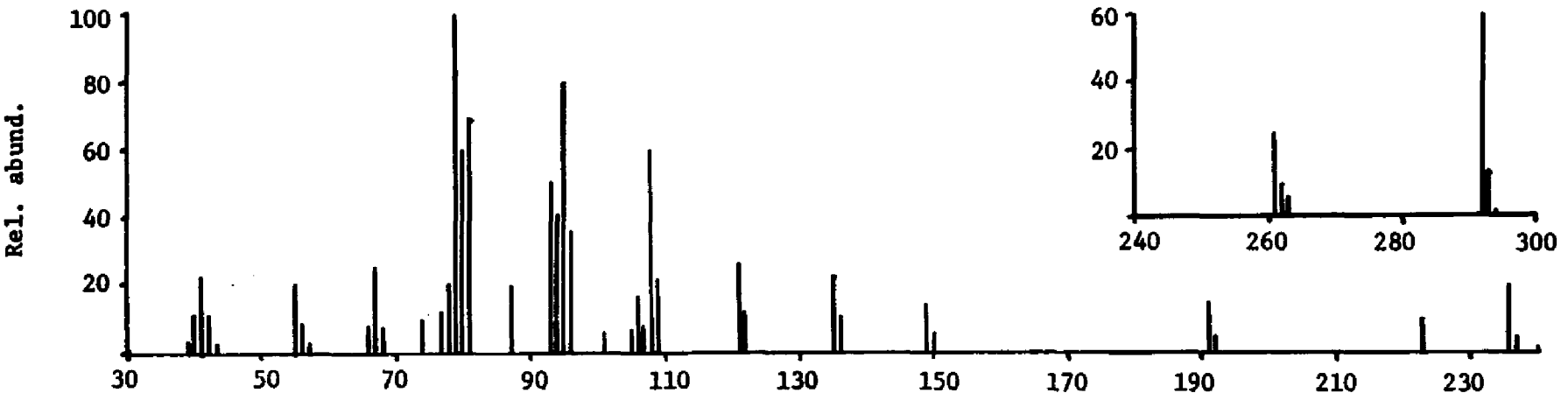

Figure No. 32

Mass Spectrum of Methyl Octadecantrienoate m/e 292

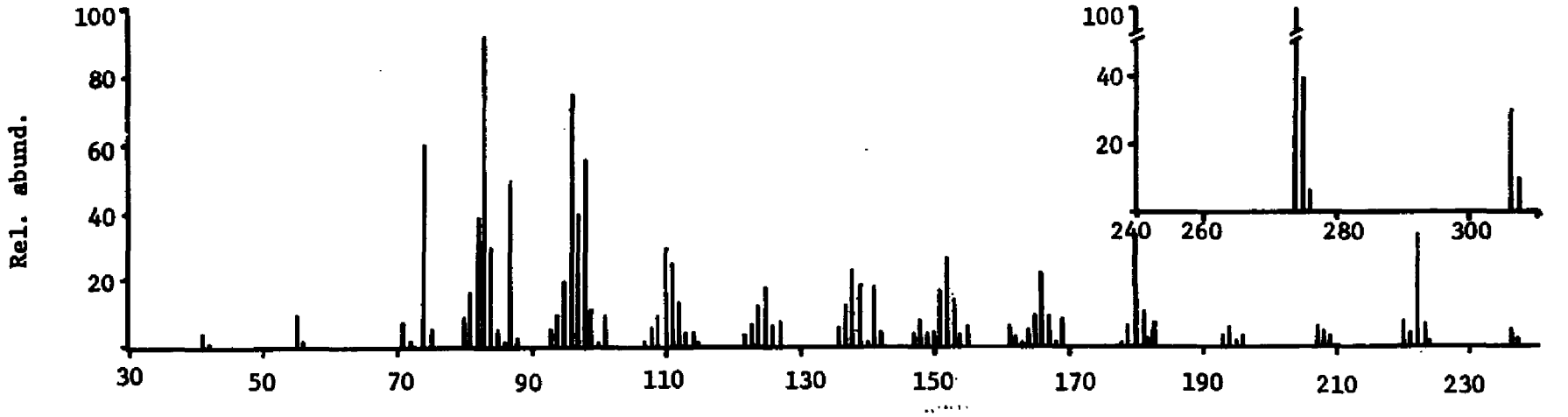

Figure No. 33

Mass Spectrum of Methyl Octadecenoate m/e 296

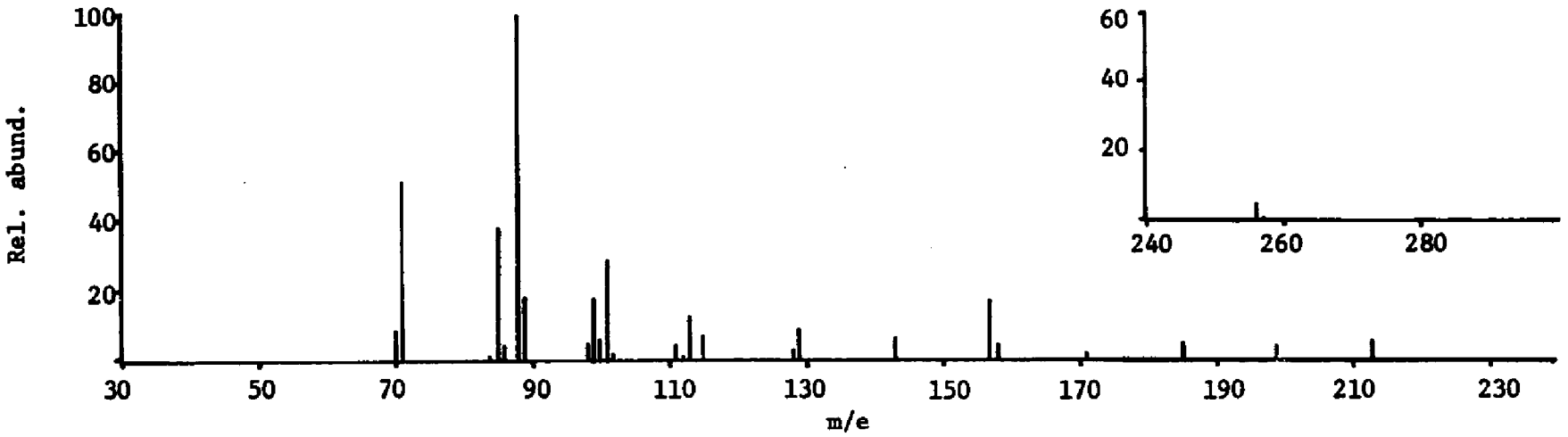

Figure No. 34

Mass Spectrum of Ethyl n-Tetradecanoate m/e 256

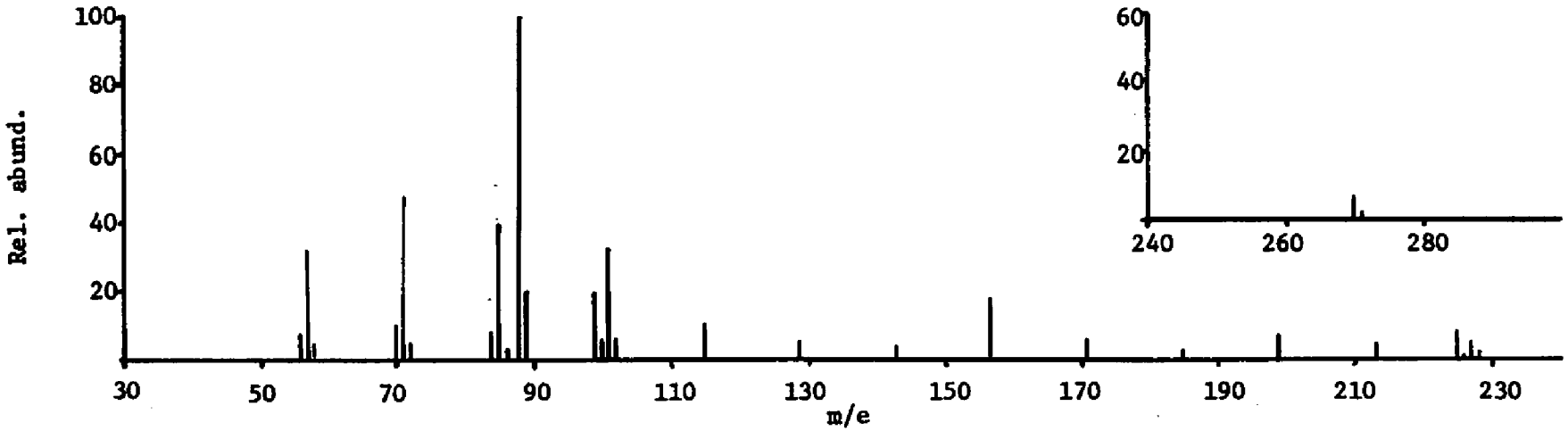

Figure No. 35

Mass Spectrum of Ethyl n-Pentadecanoate m/e 270

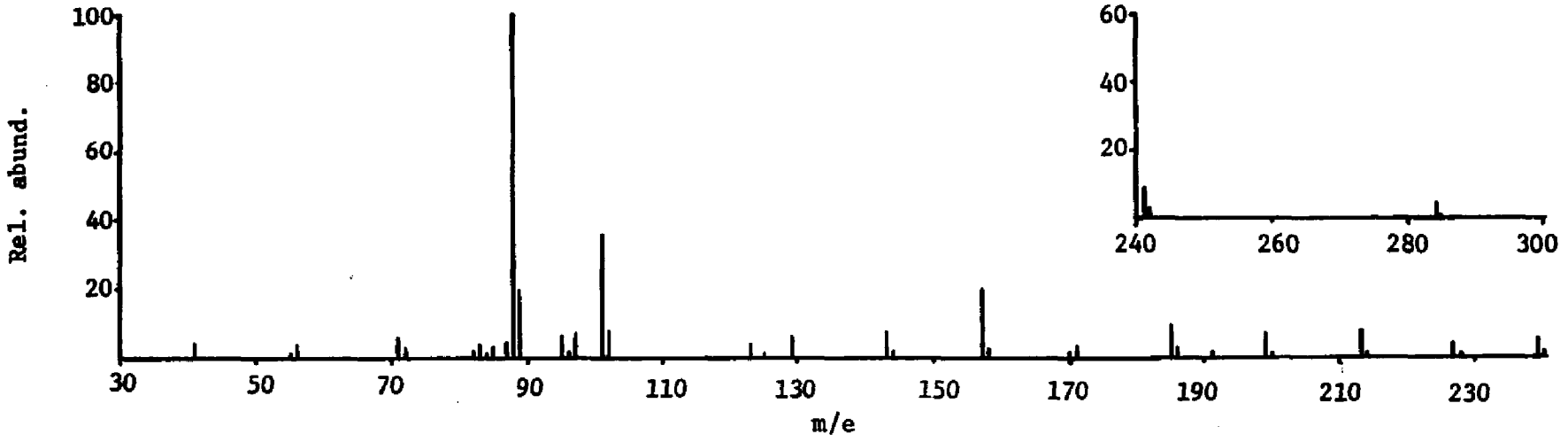

Figure No. 36

Mass Spectrum of Ethyl n-Hexadecanoate m/e 284

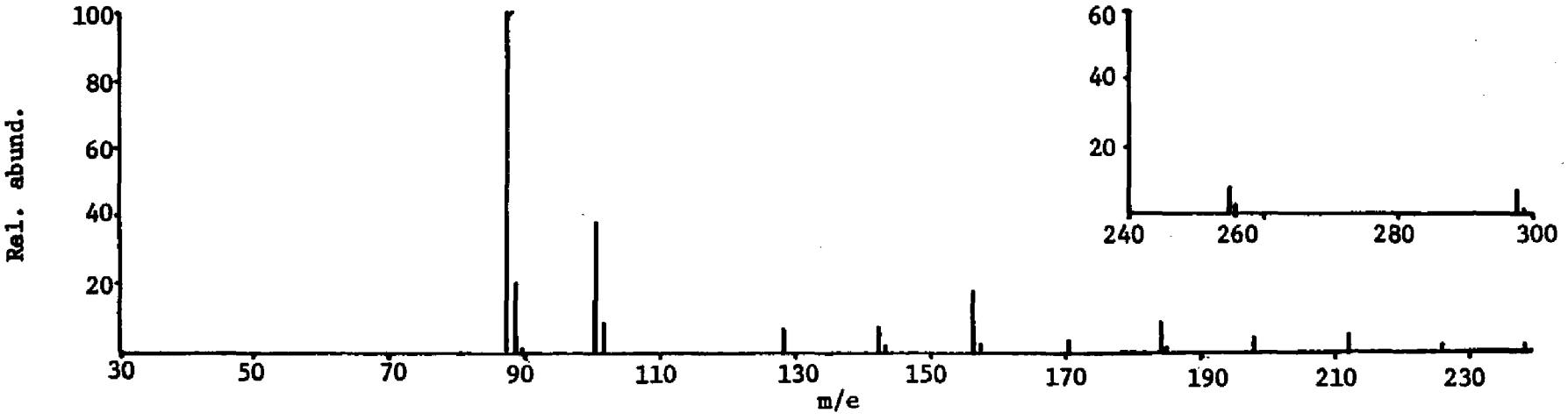

Figure No. 37

Mass Spectrum of Ethyl n-Heptadecanoate m/e 298

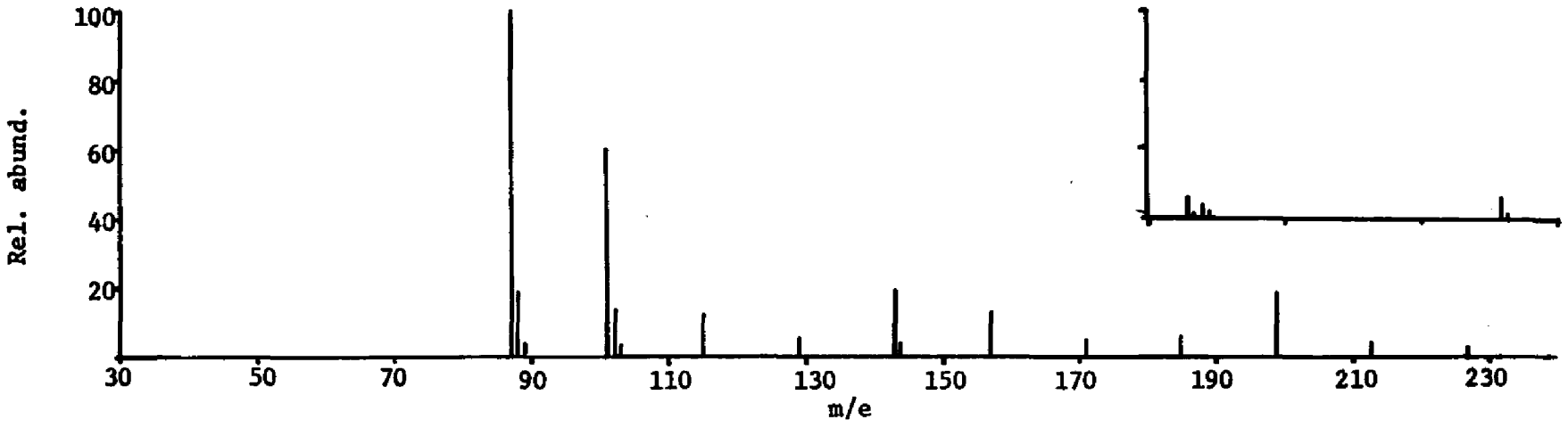

Figure No. 38

Mass Spectrum of Ethyl n-Octadecanoate m/e 312

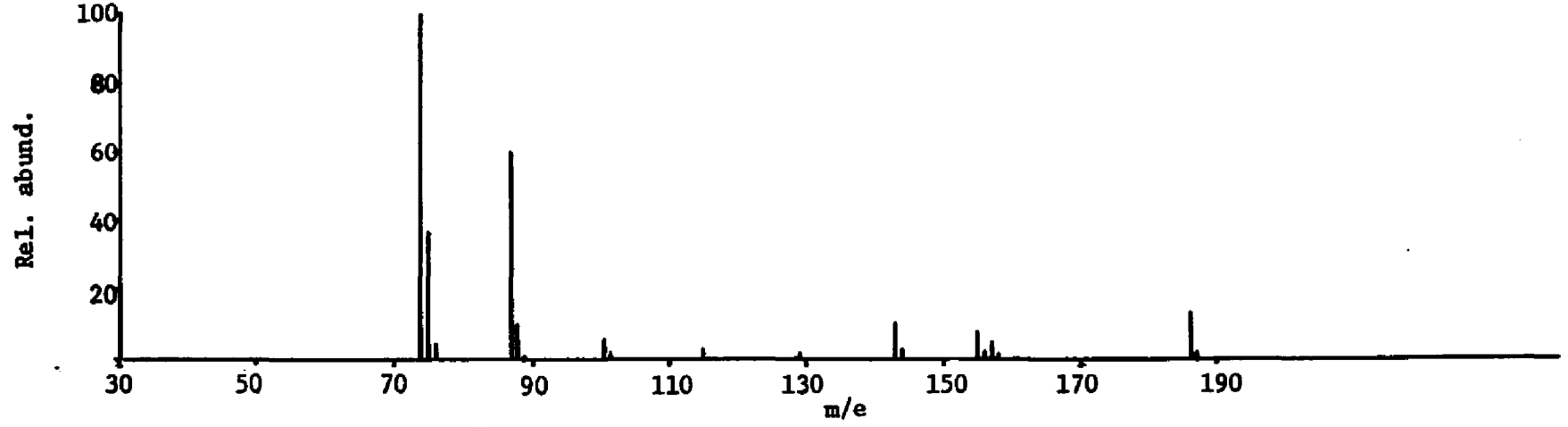

Figure No. 39

Mass Spectrum of Methyl n-Decanoate m/e 186

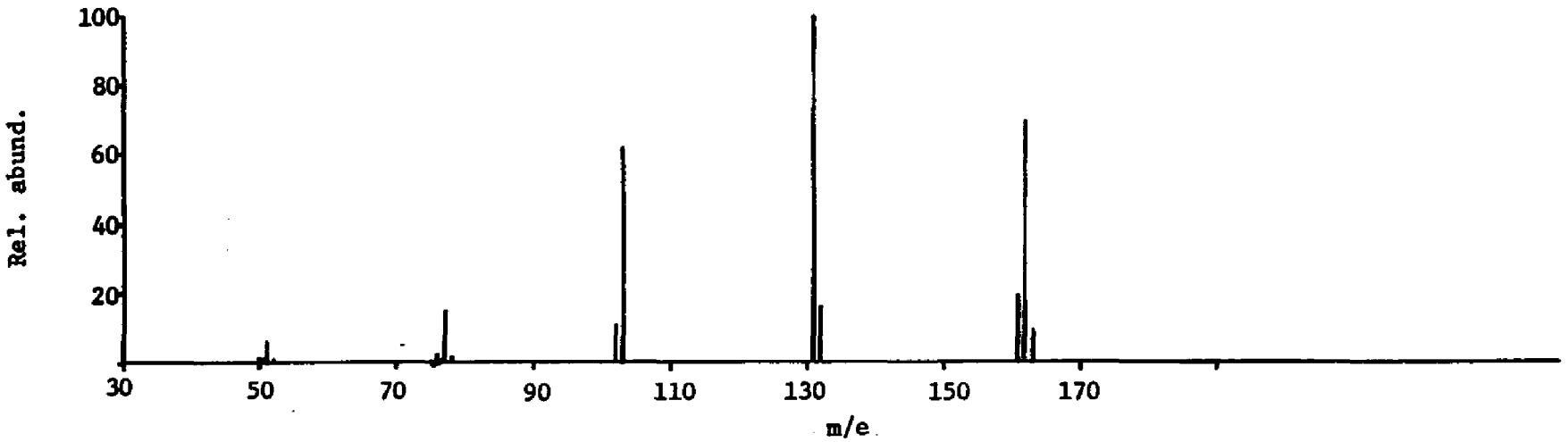

Figure No. 40

Mass Spectrum of Methyl Cinnamate m/e 162

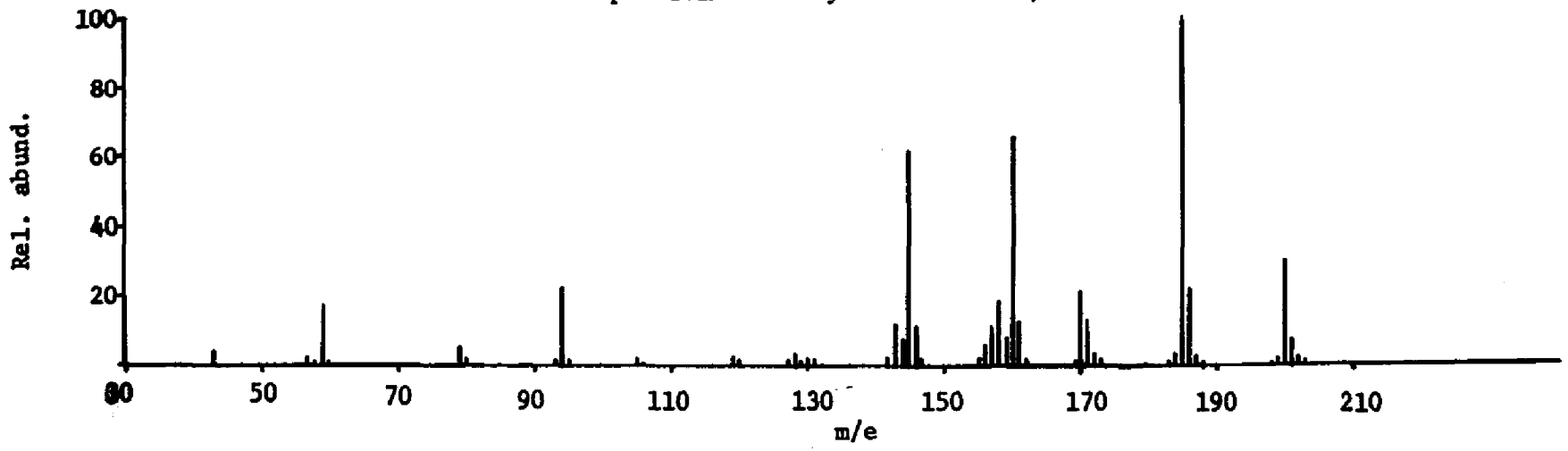

Figure No. 41

Unidentified

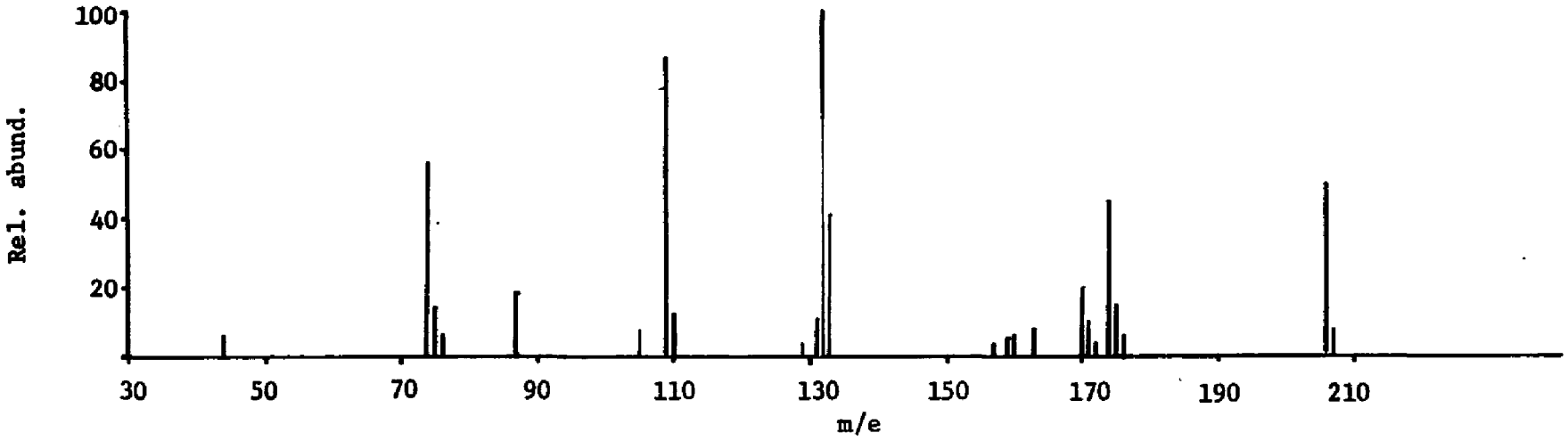

Figure No. 42

Unidentified

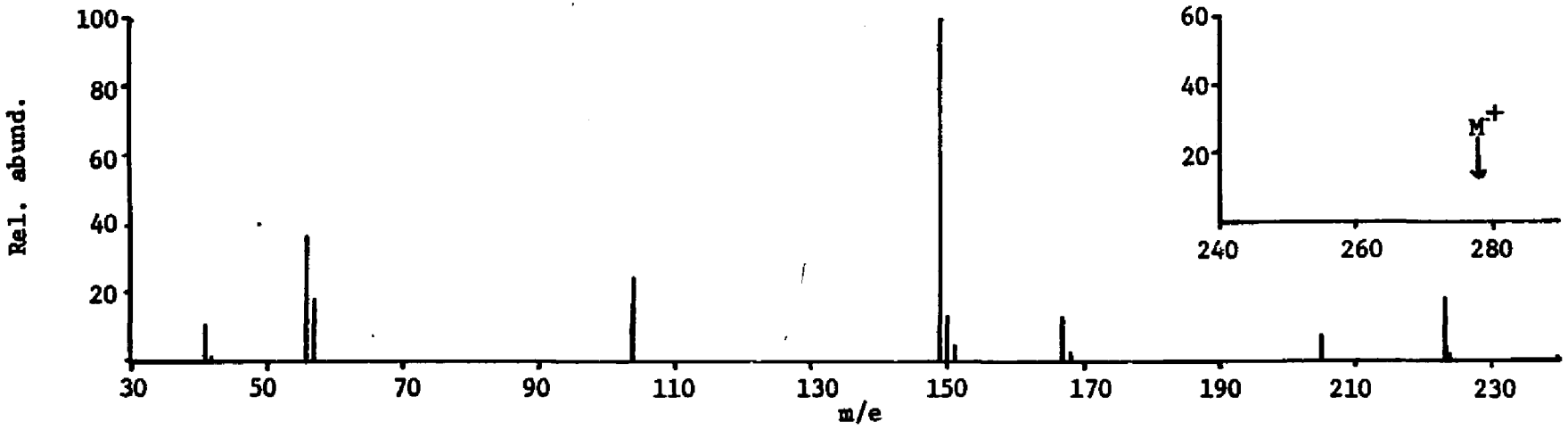

Figure No. 43

Mass Spectrum of n-Dibutyl Phthalate m/e 278

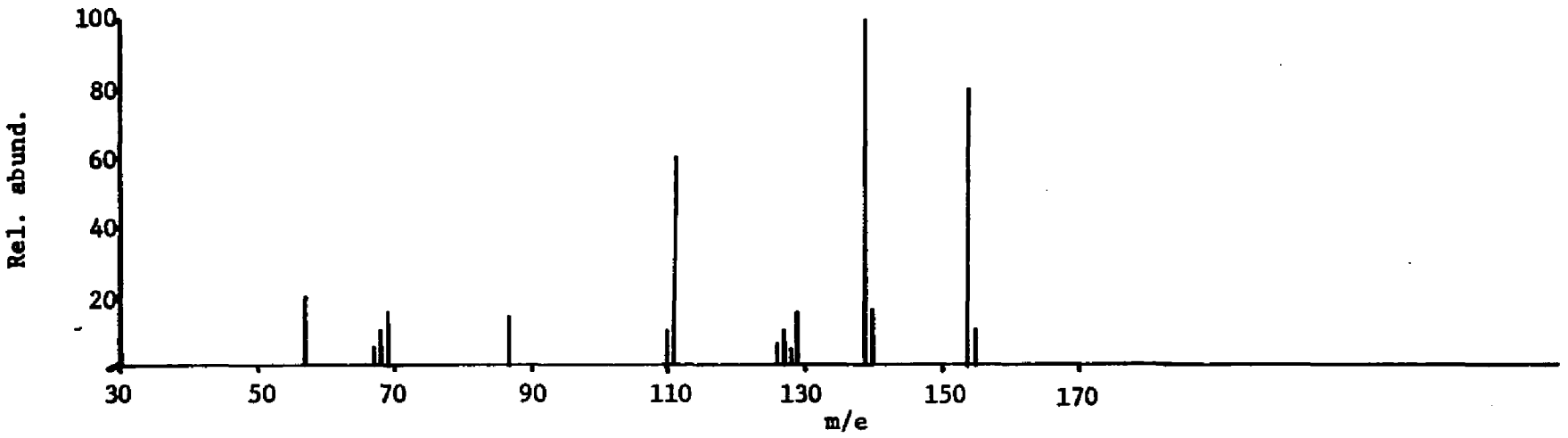

Figure No. 44

Unidentified

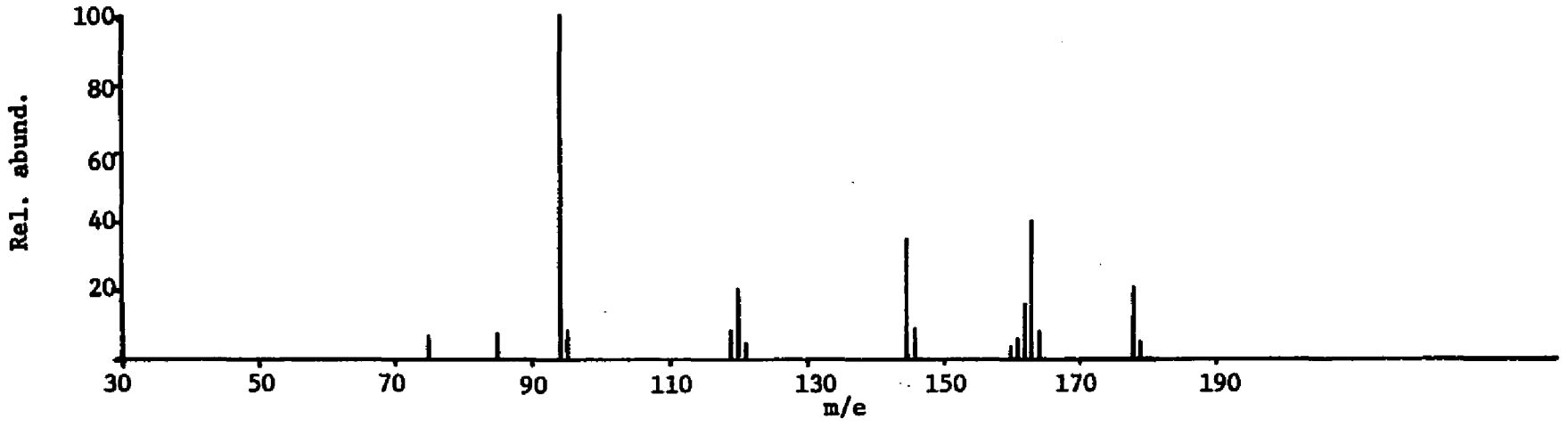

Figure No. 45

Unidentified

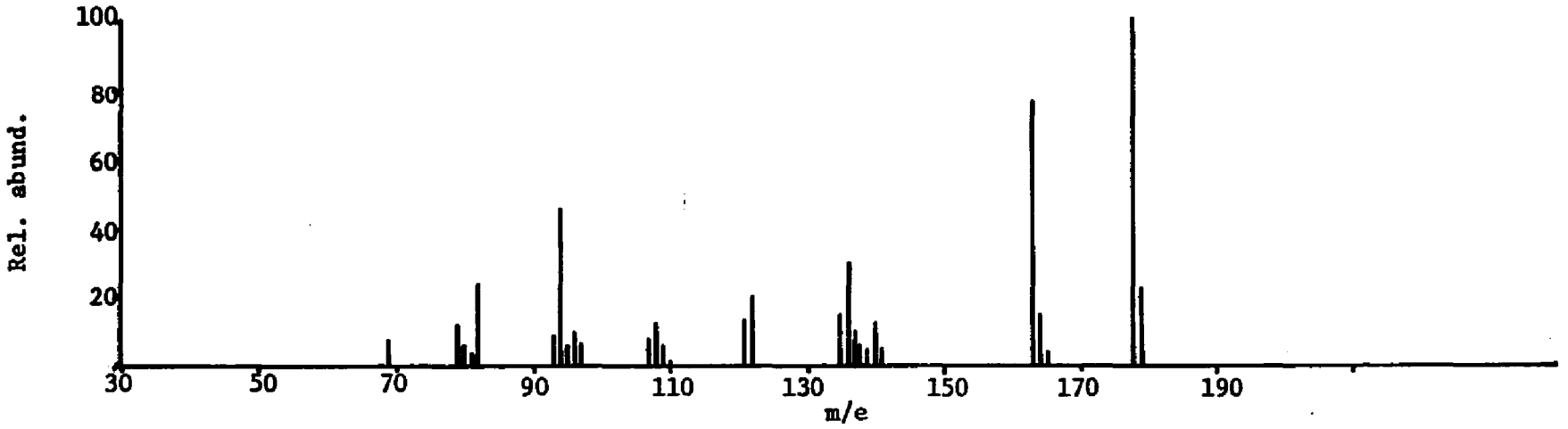

Figure No. 46

Unidentified

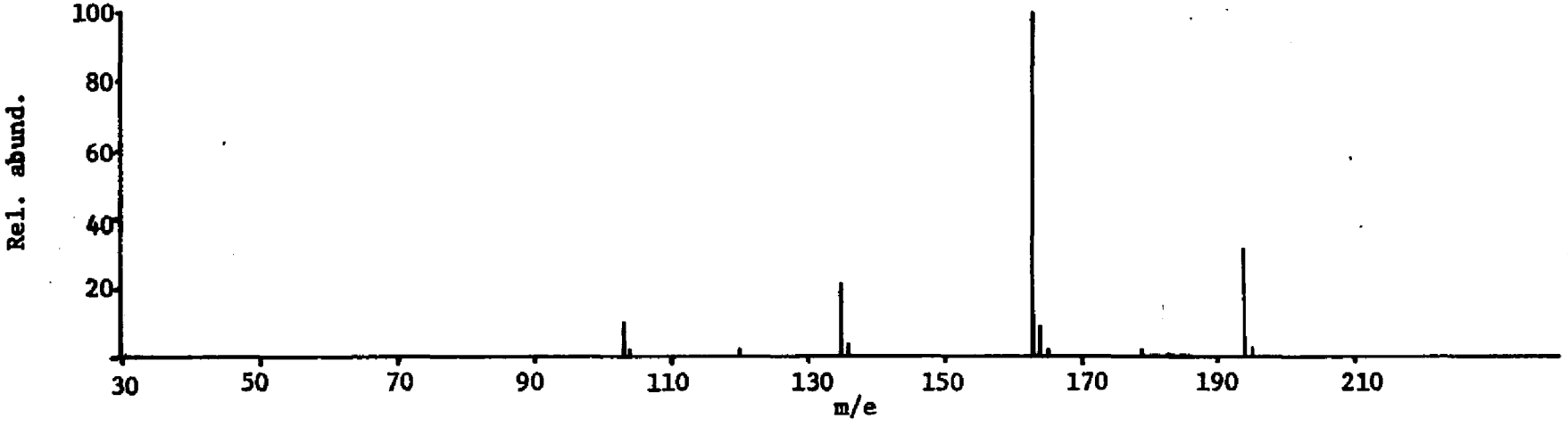

Figure No. 47

Mass Spectrum of Dimethyl Terephthalate

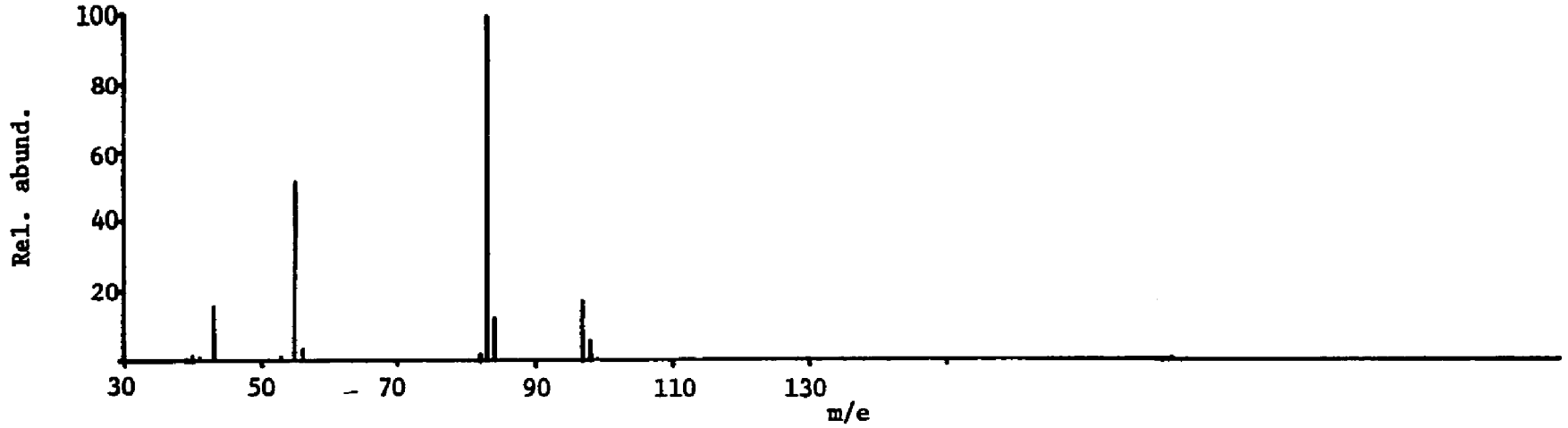

Figure No. 48

Unidentified

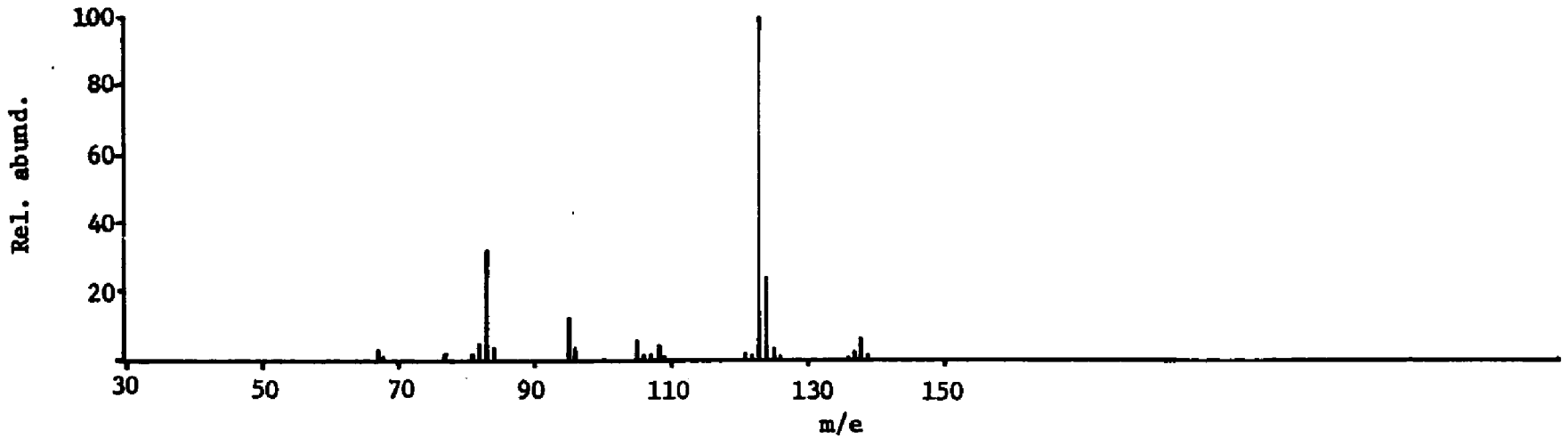

Figure No. 49

Unidentified

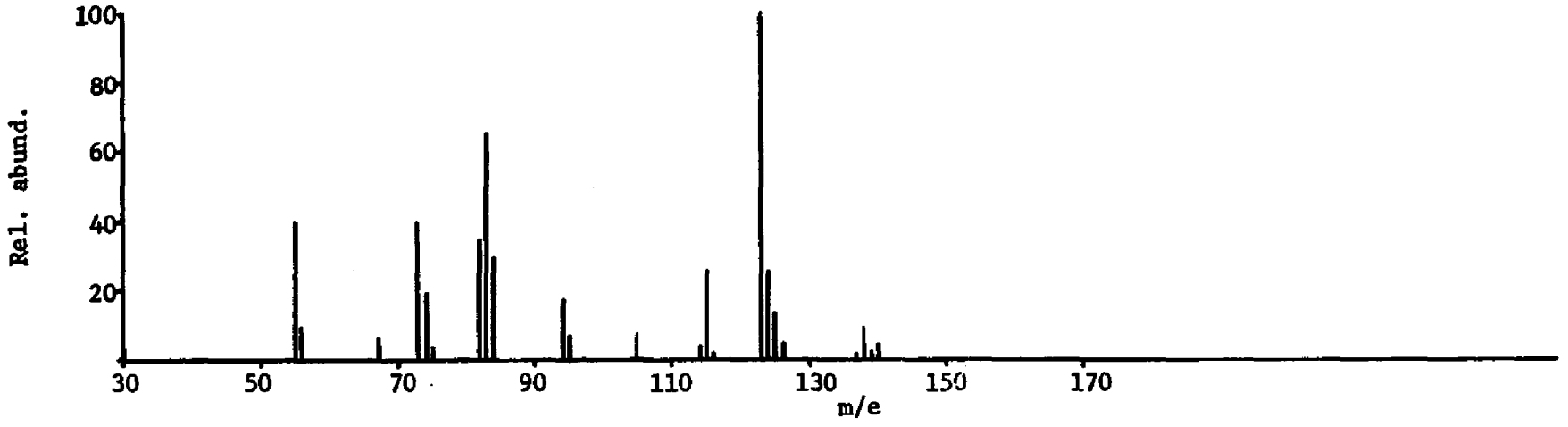

Figure No. 50

Unidentified

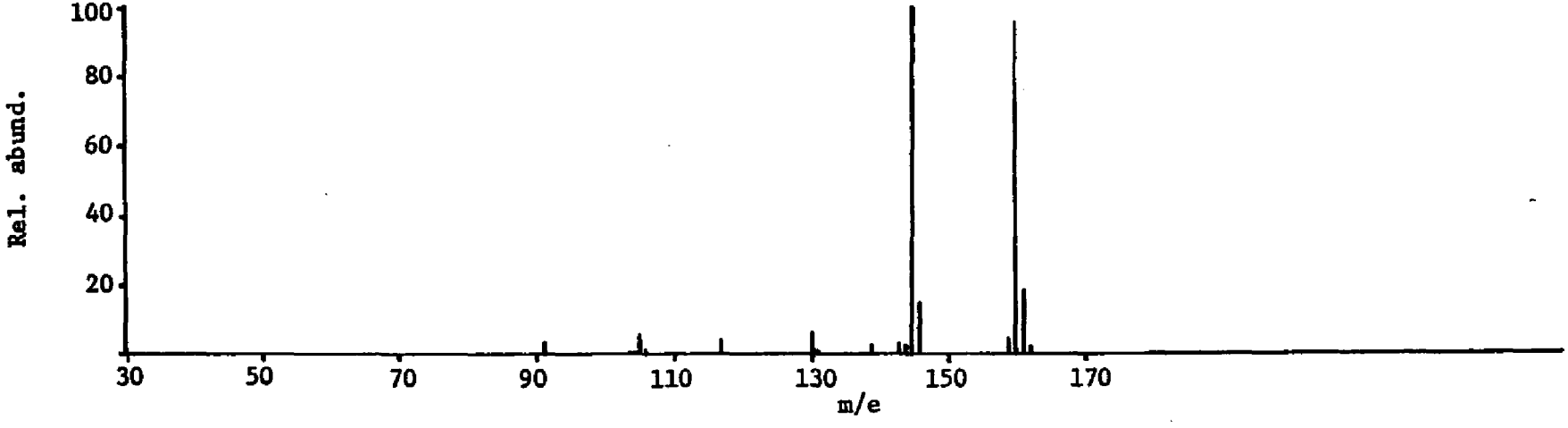

Figure No. 51

Unidentified

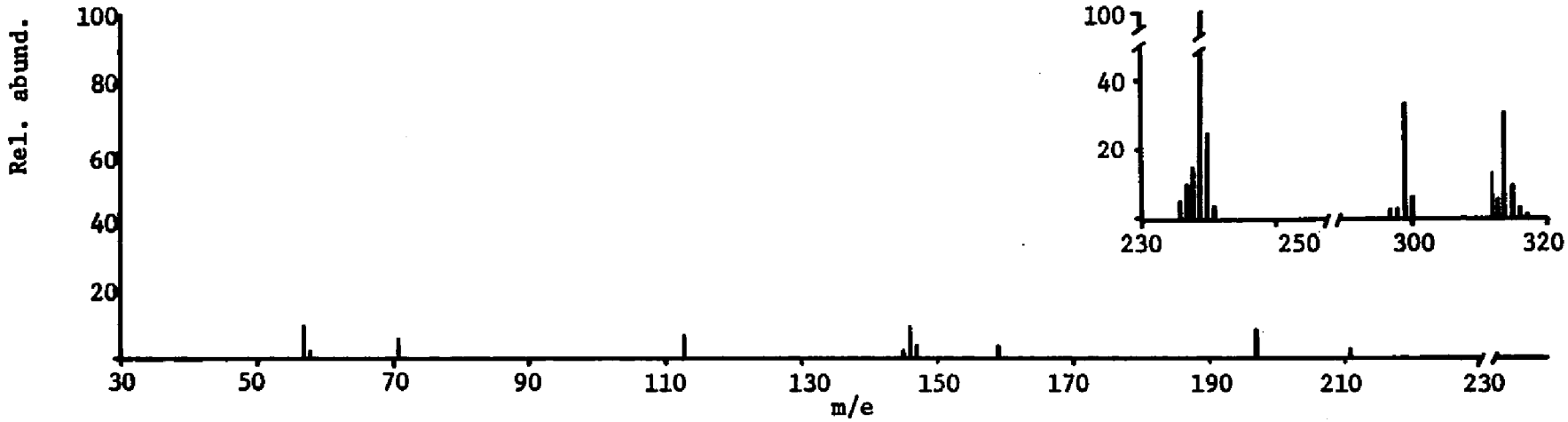

Figure No. 52

Unidentified

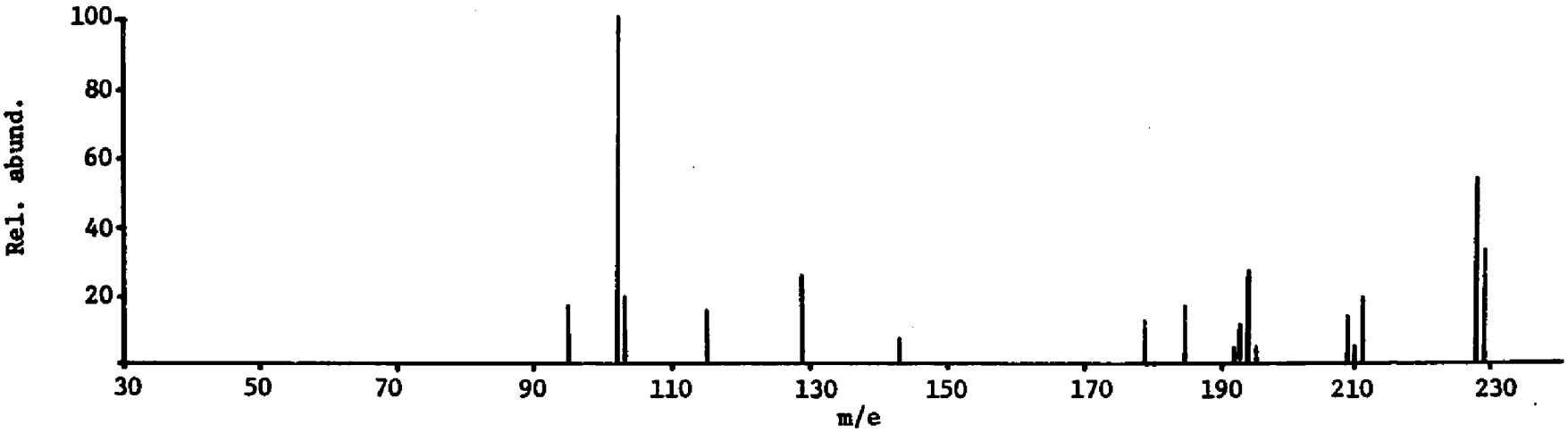

Figure No. 53

Unidentified